

## METHODS AND COMPOSITIONS FOR DESENSITISATION

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The present invention relates to methods and compositions for desensitising patients who are hypersensitive to particular allergens, especially polypeptide allergens. Moreover, the invention relates to immunological vaccines which may be used to prevent and/or treat conditions involving hypersensitivity to allergens.

The ability of the immune system to elicit a response to a particular molecule depends critically upon its ability to recognise the presence of an antigen. Classically, the term antigen has been associated with the ability of a molecule to be an antibody-generator *via* induction of B-cells. It is now known, however, that T cells also possess the ability to recognise antigens. T-cell antigen recognition requires antigen presenting cells (APCs) to present antigen fragments (peptides) on their cell surface in association with molecules of the major histocompatibility complex (MHC). T cells use their antigen specific T-cell receptors (TCRs) to recognise the antigen fragments presented by the APC. Such recognition acts as a trigger to the immune system to generate a range of responses to eradicate the antigen which has been recognised.

T lymphocytes have been implicated in the pathogenesis of a wide variety of diseases involving immune recognition of antigens derived both from the internal (host) and external environments. Autoimmune diseases such as autoimmune thyroiditis, rheumatoid arthritis and lupus erythematosus arise from the recognition by the immune system of host, or self, antigens.

such as man, can in some cases result in diseases, known as atopic conditions. An example of the latter are the allergic diseases including

asthma, atopic dermatitis and allergic rhinitis. In this group of diseases, B lymphocytes generate antibodies of the IgE class (in humans) which bind externally derived antigens, which are referred to in this context as allergens, since these molecules elicit an allergic response. Production of allergen-specific IgE is dependent upon T lymphocytes which are also activated by (are specific for) the allergen. Allergen-specific IgE antibodies bind to the surface of cells such as basophils and mast cells by virtue of the expression by these cells of surface receptors for IgE. Crosslinking of surface bound IgE molecules by allergen results in degranulation of these effector cells causing release of inflammatory mediators such as histamine, 5-hydroxytryptamine and lipid mediators such as the sulphidoleukotrienes. In addition to IgE-dependent events, certain allergic diseases such as asthma are characterised by IgE-independent events. It has been demonstrated that the induction of the late phase reaction is an IgE-independent event which is dependent upon the activation of allergen-specific T lymphocytes.

Allergic IgE-mediated diseases are currently treated with agents which provide symptomatic relief or prevention. Examples of such agents are anti-histamines,  $\beta_2$  agonists, and glucocorticosteroids. In addition, some IgE-mediated diseases are treated by desensitisation procedures that involve the periodic injection of allergen components or extracts. Desensitisation treatments may induce an IgG response that competes with IgE for allergen, or they may induce specific suppressor T cells that block the synthesis of IgE directed against allergen. This form of treatment is not always effective and poses the risk of provoking serious side effects, particularly general anaphylactic shock. This can be fatal unless recognised and treated promptly. An alternative approach is to suppress the unwanted allergic-immune response to a particular allergen, without altering the immune reactivity to

other foreign antigens or triggering an allergic response itself would be of great benefit to allergic individuals.

Asthma can be provoked by inhalation of allergen in the clinical  
5 laboratory under controlled conditions. The response is characterised by an early asthmatic reaction (EAR) followed by a delayed-in-time late asthmatic reaction (LAR) (See *Allergy and Allergic Diseases* (1997), A.B. Kay (Ed.), Blackwell Science, pp 1113 to 1130). The EAR occurs within minutes of exposure to allergen, is maximal between 10 and 15 min and  
10 usually returns to near baseline by 1 hour. It is generally accepted that the EAR is dependent on the IgE-mediated release of mast cell-derived mediators such as histamine and leukotrienes. In contrast the LAR reaches a maximum at 6-9 hours and is believed to represent, at least in part, the inflammatory component of the asthmatic response and in this sense has  
15 served as a useful model of chronic asthma.

The late asthmatic response is typical of responses to allergic stimuli collectively known as late phase responses (LPR). LPR is seen particularly in the skin and the nose following intracutaneous or intranasal  
20 administration of allergens.

Using cat allergic individuals (rhinitic and asthmatic), Norman *et al* (1996) *Am. J. Respir. Crit. Care Med.* 154:1623-8 attempted to induce the counterpart of murine experimental T cell tolerance by subcutaneous  
25 injection of "T cell reactive peptides" (termed IPC1 and IPC2) in humans. Peptides were designed on the basis of patterns of epitope recognition of short overlapping peptides by Fel d 1 reactive T cell lines. It was found

that the majority of activity being associated in the N-terminal region of chain 1. IPC1 and IPC2 were considerably longer (27

amino acids each) than previously defined T-cell epitopes. This may have been partly responsible for immediate (presumed IgE-mediated) reactions in some patients following administration (Norman *et al.*, Op. Cit.). Large peptide doses (4 x 750 µg) were required to achieve minimal clinical efficacy. The choice of peptides for therapy was based upon reactivity of secondary T-cell lines derived from a large number of cat-allergic individuals and did not take into account primary T-cell reactivity (ie *ex vivo*), which may be more sensitive, or MHC class II haplotype.

Norman *et al.* reported a number of adverse hypersensitivity reactions including respiratory, and other allergic, symptoms. As stated, some had a rapid time of onset ie with 10 minutes whereas others were not observed until several hours after IPC1/IPC2 administration (although there was no local redness or swelling at the site of injection). These results have been interpreted as indicating the unsuitability of the peptides for immunotherapy, the production of a LPR being considered to be undesirable (Wheeler & Drachenberg (1997) *Allergy* 52:602-612).

WO 92/11859 describes a method of reducing the immune response to an allergen in which a non-allergen derived, non-stimulating peptide which binds to specific MHC class II molecules of APCs is used to inhibit T-cell response to particular allergens.

WO 91/06571 purports to disclose peptides derived from human T-cell reactive feline protein which can be used in the diagnosis, treatment or prevention of cat allergy.

WO 99/34826 describes peptides which have the intent of reducing the level of undesirable side effects associated with desensitising therapies.

We have observed that peptide allergens used in immunotherapy associate with particular MHC types in patients. Moreover, successful desensitisation of patients is achieved where a peptide allergen is used which is capable of giving an initial LPR in an individual to whom it is administered.

The MHC complex is a genetic locus made up of a number of genes which encode MHC molecules. MHC molecules are also known as Human Leucocyte Antigens (HLA).

Each individual inherits a number of MHC genes from each parent and the genes are referred to collectively as the individual's haplotype. This is a genetic term referring to the genes rather than the molecules they encode. Although the term "haplotype" should, strictly speaking, be used to describe the genes inherited from one parent, it is generally used to include genes from both sets of parents. Where the term is used in this patent specification it is given this general meaning unless the context suggests the stricter meaning.

A first aspect of the invention provides a method of desensitising a patient to a polypeptide allergen the method comprising administering to the patient a peptide derived from the allergen wherein restriction to a MHC Class II molecule possessed by the patient can be demonstrated for the peptide and the peptide is able to induce a late phase response in an individual who possesses the said MHC Class II molecule.

#### Restriction to a MHC Class II molecule

The term "restriction to a MHC Class II molecule" means that the peptide is able to induce a late phase response in an individual who possesses the said MHC Class II molecule. By "MHC Class II molecule possessed by the patient" is meant

the particular type which type, of course, may be possessed by other individuals which have the genes that encode the particular type of MHC Class II molecule.

- 5 By a "peptide derived from the allergen" we include the meaning that the peptide is chemically derived from the polypeptide allergen, for example by proteolytic cleavage and we also include the meaning that the peptide is derived in an intellectual sense from the polypeptide allergen, for example by making use of the amino acid sequence of the polypeptide allergen and  
10 synthesising peptides based on the sequence. Peptides may be synthesised using methods well known in the art, some of which are described in more detail below.

- By "peptide" we include not only molecules in which amino acid residues  
15 are joined by peptide (-CO-NH-) linkages but also molecules in which the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Mézière *et al* (1997) *J. Immunol.* 159, 3230-3237, incorporated herein by reference. This approach involves making pseudopeptides containing  
20 changes involving the backbone, and not the orientation of side chains. Mézière *et al* (1997) show that, at least for MHC class II and T helper cell responses, these pseudopeptides are useful. Retro-inverse peptides, which contain NH-CO bonds instead of CO-NH peptide bonds, are much more resistant to proteolysis.

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Similarly, the peptide bond may be dispensed with altogether provided that an appropriate linker moiety which retains the spacing between the Ca

- 20 substantially the same planarity as a peptide bond

It will be appreciated that the peptide may conveniently be blocked at its N- or C-terminus so as to help reduce susceptibility to exoproteolytic digestion.

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By "restriction to a MHC Class II molecule possessed by the patient can be demonstrated for the peptide" we mean that the peptide is able to bind to a particular MHC Class II possessed by the patient. That is not to say that a particular peptide cannot bind to another MHC Class II molecule.

10 Peptides are generally only recognised in the context of a "self" MHC molecule, thus recognition of MHC-bound peptides by an individual's T cells is generally restricted by the MHC molecules expressed by the individual molecule.

15 Although binding to the given MHC Class II molecule may be demonstrated directly using suitable samples from the patient, whether or not a particular peptide can bind to a particular MHC Class II molecule (ie is restricted by a particular Class II molecule) can readily be determined *in vitro* using methods well known in the art, some of which are disclosed  
20 below.

Determination of the MHC Class II haplotype of the patient or the identification of particular MHC Class II genes possessed by the patient can readily be determined using any suitable method as is well known in  
25 the art, including the PCR-based methods described more fully below for example techniques based on those of Olerup & Zetterquist (1992) *Tissue Antigens* 29:225-235. Determination of the MHC Class II

By "late phase response" we include the meaning as set forth in *Allergy*

and *Allergic Diseases* (1997) A. B. Kay (Ed.), Blackwell Science, pp 1113- 1130. The late phase response may be any late phase response (LPR). Preferably, the peptide is able to induce a late asthmatic response (LAR) or a late rhinitic response, or a late phase skin response or a late phase ocular response. Whether or not a particular peptide can give rise to a LPR can be determined using methods well known in the art; a particularly preferred method is that described in Cromwell O, Durham SR, Shaw RJ, Mackay J and Kay AB. Provocation tests and measurements of mediators from mast cells and basophils in asthma and allergic rhinitis. In: *Handbook of Experimental Immunology* (4) Chapter 127, Editor: Weir DM, Blackwell Scientific Publications, 1986. Not all individuals who possess the particular MHC Class II molecule would experience a LPR following the administration of allergen or allergen-derived peptides since generation of the LPR is dependent upon prior allergic sensitisation to the allergen in question.

Thus, preferably, the peptide is able to induce a LPR in an individual who possesses the said MHC Class II molecule and who has been sensitised to the allergen in question. Whether or not an individual has been sensitised to the allergen in question may be determined by well known procedures such as skin prick testing with solutions of allergen extracts, induction of cutaneous LPRs, clinical history, allergen challenge and radio-allergosorbent test (RAST) for measurement of allergen specific IgE.

Preferably, the peptide is included in a composition containing a plurality of peptides derived from the said allergen. The peptides in the composition may or may not be multiple overlapping.

Preferably, the peptides span the whole of the polypeptide allergen and therefore the peptides span the whole of the polypeptide chain or chains of the allergen.



However, they may be derived from only portions of the polypeptide allergen such that some portions of the polypeptide allergen are not represented in the plurality of peptides (for example, as is shown below, some peptides derived from an allergen may not be very soluble in aqueous solution and so may not be useful and other peptides may not show restriction to MHC Class II molecules). MOPs or any peptides derived from the allergen and present in the composition can be designed by reference to the amino acid sequence of the polypeptide allergen. Typically, the peptides are at least seven amino acid residues. Typically, the peptides would be between around 14 to 18 amino acid residues in length. It is preferred that the peptides have a reduced ability to bind IgE compared to longer peptides containing the same sequence. It is particularly preferred if the peptides are substantially incapable of binding IgE. Typically, when the MOPs overlap, the overlap is around one amino acid residue. This is particularly useful when the MOPs are used in *in vitro* T cell assays in order to identify MHC-binding peptides which may then be screened for their ability to induce LPR in an individual. More details of screening procedures are given below.

MHC Class II molecules are encoded by MHC Class II genes. There are at least three loci (DR, DQ and DP) that encode MHC Class II molecules, and each individual has two copies of each locus. These loci exhibit considerable genetic diversity and the preponderance of different MHC Class II genes (alleles) varies. The approximate frequencies of various MHC Class II genes (alleles) from a normal (disease free) population of people in England is described in Haworth S, Sinnott P, Davidson J & Dyer P. Caucasian England. *Manuscript*.

laboratory, incorporated herein by reference

For DR molecules, the most common in the Caucasian population are those that can be classified DR1, DR2, DR3, DR4, DR5, DR6, DR7, DR51, DR52 and DR53.

- 5 For DP molecules, the most common are DPB1\*0201, DPB1\*0301 and DPB1\*0401.

For DQ molecules, the most common are DQB1\*0201, DQB1\*0301, DQB1\*0501, DQB1\*0601 and DQB1\*0602.

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It is particularly preferred if the plurality of polypeptides administered to the patient includes peptides for which restriction to MHC Class II molecules can be demonstrated. It is particularly preferred if the plurality of peptides administered to the patient includes peptides for which  
15 restriction to the MHC Class II DR molecules DR2, DR3, DR4, and DR7 can be demonstrated. In a further embodiment it is preferred if the plurality of peptides further includes peptides for which restriction to any one or more of the MHC Class II DR molecules DR1, DR5 and DR6 can be demonstrated.

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It is also particularly preferred if the plurality of peptides administered to the patient includes peptides for which restriction to the MHC Class II DR molecules DR51, DR52 and DR53 has been demonstrated.

- 25 It is also particularly preferred if the plurality of peptides administered to the patient includes peptides for which restriction to the MHC Class II DP molecules DPB1\*0201, DPB1\*0301 and DPB1\*0401 has been demonstrated.

30 It is also particularly preferred if the plurality of peptides administered to

the patient includes peptides for which restriction to the MHC Class II DQ molecules DQB1\*0301 and DQB1\*0601 can be demonstrated. In a further embodiment it is preferred if the plurality of peptides further includes peptides for which restriction to any one or more of the MHC Class II DQ molecules DQB1\*0201, DQB1\*0501 and DQB1\*0602 can be demonstrated.

It is preferred if the plurality of peptides includes only a single peptide for which restriction to a particular MHC Class II molecule can be demonstrated.

Restriction to a particular Class II molecule can be demonstrated as has been described above and is described in more detail below. It will be appreciated that it may not be possible to derive a peptide for which restriction to a particular Class II molecule can be demonstrated; for example, a particular polypeptide allergen may not contain a T cell epitope which can be presented by every MHC Class II molecule. In this case, of course, such a peptide is not present in the plurality of peptides derived from the polypeptide allergen.

By "desensitising a patient to a polypeptide allergen" is meant inhibition or dampening of allergic tissue reactions induced by allergens in appropriately sensitised individuals. It will be appreciated that whether or not a patient is sensitive to a particular polypeptide allergen can be assessed using well known procedures such as skin prick testing with solutions of allergen extracts, induction of cutaneous LPRs, clinical history, allergen challenge and radioallergy tests.

One who is expected to benefit from treatment may be determined by the physician based, for example, on such tests.

Administration of the peptide (such as the composition containing a plurality of peptides) may be by any suitable method, some of which are described below in more detail. Suitable amounts of the peptide may be determined empirically, but typically are in the range given below. As is  
5 described in a further aspect of the invention below, the invention also includes a method of determining an initial dose of peptide which is suitable to administer to the patient. A single administration of the peptide may be sufficient to have a beneficial effect for the patient, but it will be appreciated that it may be beneficial if the peptide is administered more  
10 than once, in which case typical administration regimes may be, for example, once or twice a week for 2-4 weeks every 6 months, or once a day for a week every four to six months.

A second aspect of the invention provides a composition comprising a  
15 plurality of peptides derived from a polypeptide allergen wherein for at least one of the peptides in the composition restriction to a MHC Class II molecule can be demonstrated and the composition is able to induce a late phase response in an individual possessing the given MHC Class II molecule. Preferably, at least one peptide is present in the composition  
20 for which restriction to each of MHC Class II DR molecules DR2, DR3, DR4 and DR7 can be demonstrated, provided of course that such peptides can be derived from the allergen.

Also preferably the composition may include peptides for which restriction  
25 to any one or more of the MHC Class II DR molecules DR1, DR5 and DR6 can be demonstrated.

Restriction to each of MHC Class II DR molecules DR51, DR52 and  
30 DR53 has been demonstrated.

Preferably, at least one peptide is present in the composition for which restriction to each of MHC Class II DP molecules DPB1\*0201, DPB1\*0301, and DPB1\*0401 can be demonstrated.

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Preferably, at least one peptide is present in the composition for which restriction to each of MHC Class DQ molecules DQB1\*0301 and DQB1\*0601 can be demonstrated. In a further embodiment it is preferred if the plurality of peptides further includes peptides for which restriction to  
10 any one or more of the MHC Class II DQ molecules DQB1\*0201, DQB1\*0501 and DQB1\*0602 can be demonstrated.

These preferences are all with the proviso that for any particular allergen it may not be possible to derive a peptide for which restriction to a  
15 particular Class II molecule can be demonstrated.

Although the composition (or a peptide within the composition) is able to induce a LPR in an individual possessing the given MHC Class II molecule (and as described below in more detail suitable compositions and  
20 peptides may be identified by their ability to induce a LPR), it should be appreciated that when the composition (or a peptide within the composition) is used to treat a patient it is preferable that a sufficiently low concentration of the composition or peptide is used such that no observable LPR will occur but the response will be sufficient to partially  
25 desensitise the T cells such that the next (preferably higher) dose may be given, and so on. In this way the dose is built up to give full desensitisation but often with

at a higher concentration than is administered. It will be appreciated further,  
30 and as discussed in more detail below, induction of LPR in an individual

is particularly useful in selecting appropriate compositions and peptides but is not essential in the clinical efficacy and treatment stages.

It will be appreciated that the composition may contain as many or as few peptides derived from the polypeptide allergen as will make it useful. Although in one embodiment of the method of desensitising the patient of the first aspect of the invention a single peptide may be administered to the patient wherein the peptide demonstrates restriction to a MHC Class II molecule possessed by the patient and the peptide is able to induce a late phase response in an individual who possesses the said MHC Class II molecule, it is preferred if the composition of the second aspect of the invention contains sufficient number of peptides, each of which demonstrate restriction to a particular MHC Class II molecule and which are able to induce a late phase response in an individual who possesses the said MHC Class II molecule, such that for at least 75% of the population a peptide is present in the composition which is MHC Class II restricted and which is capable of inducing a late phase response in an individual with an appropriate restricted MHC Class II molecule. More preferably the composition contains sufficient peptides such that for at least 80% of the population (and still more preferably at least 85%, or yet still more preferably 90% of the population) a peptide is present in the composition which is MHC Class II restricted and which is capable of inducing a late phase response in an individual with an appropriate restricted MHC Class II molecule.

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In a particularly preferred embodiment, the composition contains (as the only polypeptide allergen-derived peptide)

inducing a LPR in an individual who possesses the given MHC Class II molecule. Preferably, the composition contains as the only polypeptide

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allergen-derived peptide components a sufficient number of peptides, each of which demonstrate restriction to a particular MHC Class II molecule and which are able to induce a LPR in an individual who possesses the said MHC Class II molecule, such that for at least 75% of the population a peptide is present in the composition which is MHC Class II restricted and which is capable of inducing a LPR in an individual with an appropriate restricted MHC Class II molecule.

It is well known that the frequency of particular MHC Class II molecules in a population varies with ethnic groups, and that for at least some ethnic groups the frequency of particular MHC Class II molecules is known (see, for example, HLA Typing 1997, supra). For example, the frequency of particular MHC Class II molecules is different in the Caucasian population compared to the Mongoloid population or Negroid population and so on. It will readily be appreciated that the polypeptide allergen-derived peptides to be included in a composition of the invention may be selected according to the ethnic group to which the patient belongs. For example, compositions of the invention may readily be prepared for desensitisation to a particular polypeptide allergen by reference to the MHC Class II gene frequencies in the Caucasian or Mongoloid or Negroid populations.

A third aspect of the invention provides a composition of the second aspect of the invention packaged and presented for use in medicine. In particular, the composition will be packaged and presented with an indication of who may be treated (in particular who may benefit from being treated) with the composition including, if desirable, an indication

It will be appreciated that the composition of the invention may be

invention is conveniently administered to the patient according to the method of the first aspect of the invention.

5 A fourth aspect of the invention provides a pharmaceutical formulation comprising a composition according to the second aspect of the invention and a pharmaceutically acceptable carrier. Suitable ingredients for pharmaceutical formulations are described in more detail below.

10 A fifth aspect of the invention provides the use of a peptide derived from a polypeptide allergen wherein restriction to a MHC Class II molecule possessed by a patient can be demonstrated for the peptide and the peptide is able to induce a late phase response in an individual who possesses the said MHC Class II molecule in the manufacture of a medicament for desensitising a patient to said polypeptide allergen.

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A sixth aspect of the invention provides the use of a composition according to the second aspect of the invention in the manufacture of a medicament for desensitising a patient to said polypeptide allergen.

20 It will be appreciated that with respect to the method of the first aspect of the invention it may be desirable to determine which MHC Class II molecules the patient possesses in order to select an appropriate peptide or composition to administer to the patient. (It will be appreciated that this may be determined by determining the MHC haplotype of the individual by genetic means.) This is particularly desirable when the administration  
25 of a single peptide is contemplated. However, it will also be appreciated that when a composition is used, it is desirable to select a composition which contains a peptide or peptides which are able to induce a late phase response in an individual who possesses the said MHC Class II molecule, such that for at

30 a composition is selected which contains a peptide or peptides which are able to induce a late phase response in an individual who possesses the said MHC Class II molecule, such that for at



least 75% (or more preferably 80%, or 85% or 90%) of the population a peptide is present in the composition which is MHC Class II restricted and which is capable of inducing a late phase response in an individual with an appropriate restricted MHC Class II molecule, then it may not be  
5 necessary or desirable to type the patient to determine which MHC Class II molecules he or she possesses.

The polypeptide allergen may be any polypeptide allergen, some of which are described in more detail below.

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A seventh aspect of the invention provides a method of selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to a polypeptide allergen capable of eliciting an allergic response in the patient, which patient possesses a particular MHC Class II molecule, the method  
15 comprising the steps of (1) selecting a candidate peptide derived from the polypeptide allergen, (2) determining whether the candidate peptide demonstrates restriction to the said MHC Class II molecule, and (3) determining whether the candidate peptide is able to induce a late phase response in an individual who possesses the said MHC Class II molecule.

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The candidate peptide may be any peptide derived from the polypeptide allergen and is, conveniently, a polypeptide in the size range described elsewhere as being a suitable size of a peptide for use in immunotherapy.

25 Whether or not the candidate demonstrates restriction to the said MHC Class II molecule may be determined by any suitable method such as that described in Example 1.

Whether or not the candidate peptide is able to induce a LPR can be  
30 determined by the methods described herein and which are well known in the art.

the art. It is particularly preferred if step (2) is carried out prior to step (3) and only candidate peptides which demonstrate restriction to the particular MHC Class II molecules are selected for testing in step (3).

- 5 It is particularly preferred that the individual in step (3) is an appropriately sensitised individual; that is to say an individual who has been sensitised previously to the allergen in question. It is those peptides which are capable of inducing a LPR and which demonstrate restriction to the particular MHC Class II molecule which are selected as an immuno-  
10 therapeutic agent.

Determination of whether the candidate peptide demonstrates restriction to the said MHC Class II molecules may conveniently be done using a suitable T cell activation assay.

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- Thus, in one preferred embodiment the invention provides a method for selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to an allergen capable of eliciting an allergic response in the patient which patient possesses a particular MHC Class II haplotype,  
20 comprising the steps of:

- a) administering a candidate peptide to an individual who possesses the same said MHC Class II molecule as the patient and determining whether the peptide induces a late phase response; and  
25  
b) selecting a peptide capable of inducing a late-phase response as an immunotherapeutic agent

The individual to whom the candidate peptide is administered for the purpose of determining whether the peptide induces a LPR may or may  
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not be the patient.

In an eighth aspect, the invention provides a method for testing for candidate peptides for further selection according to the preferred  
5 embodiment discussed immediately above of the invention, comprising the steps of:

- a) assaying a peptide or peptides in a T-cell activation assay and selecting peptides capable of inducing activation of an individual's T-cells;
- 10 b) tissue-typing the individual to determine MHC type;
- c) determining the MHC molecule(s) bound by each candidate peptide; and
- 15 d) selecting a peptide or peptides satisfying part (a) above and capable of binding to an MHC type possessed by the individual, for use as a candidate peptide in a method according to the preferred embodiment discussed immediately above.

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In a ninth aspect, the invention provides a method for selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to an allergen comprising the steps of:

- 25 a) tissue-typing the patient to determine MHC Class II type; and

b) administering to the patient MHC Class II molecules and induce a late phase response in an individual possessing such MHC Class II molecules, one or more peptides  
30 capable of binding to the MHC Class II molecules.

individual.

Preferably, the individual is an appropriately sensitised individual who has been sensitised previously to the allergen in question.

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In a tenth aspect, the invention provides a database of peptides characterised according to the seventh and eighth aspects of the invention.

TCRs are highly variable in their specificity. Variability is generated, as  
10 with antibody molecules, through gene recombination events within the cell. TCRs recognise antigen in the form of short peptides bound to molecules encoded by the genes of the Major Histocompatibility Complex (MHC). These gene products are the same molecules that give rise to "tissue types" used in transplantation and are also referred to as Human  
15 Leukocyte Antigen molecules (HLAs) which terms may be used interchangeably within this document. Individual MHC molecules possess peptide binding grooves which, due to their shape and charge are only capable of binding a limited group of peptides. The peptides bound by one MHC molecule may not necessarily be bound by other MHC molecules.  
20 As a result of this restricted peptide-MHC binding, T cell receptor recognition of a particular peptide is said to be "restricted" by the MHC molecule to which the peptide is bound. As used herein the term "allergen peptide-binding MHC" will be used to mean the MHC molecule(s) that bind the said allergen or allergen-derived peptide.

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When a protein molecule such as an antigen or allergen is taken up by an antigen presenting cell (APC),

and macromolecules are degraded within the cell.

The process of degradation gives rise to peptide fragments of the molecule  
30 which, if they are of the appropriate size, charge and shape, may then

bind within the peptide binding groove of certain MHC molecules and be subsequently displayed upon the surface of antigen presenting cells. If the peptide/MHC complexes are present upon the antigen presenting cell surface in sufficient numbers they may then activate T cells which bear the appropriate peptide/MHC-specific T cell receptors.

Due to the polymorphic nature of the MHC, individuals in an outbred population such as man will express different combinations of MHC molecules on their cell surfaces. Since different MHC molecules can bind different peptides from the same molecule based on the size, charge and shape of the peptide, different individuals will display a different repertoire of peptides bound to their MHC molecules.

Identification of universal MHC-binding peptide epitopes in an outbred population such as man is more difficult than in inbred animals (such as certain strains of laboratory mice). On the basis of differential MHC expression between individuals and the inherent differences in peptide binding and presentation which this brings, it is unlikely that a single peptide can be identified which will be of use for desensitisation therapy in man for most diseases unless the association of a particular MHC molecule with that disease is very strong. For example, the HLA-B27 molecule has been shown to have a close relationship with ankylosing spondylitis, where approximately 90% of sufferers express HLA-B27. For some autoimmune diseases, certain disease HLA associations have been demonstrated eg HLA-DR4 and rheumatoid arthritis, but these associations are much weaker than for ankylosing spondylitis.

For this reason, it is unlikely that therapies centred around a single peptide (even an immunodominant one) or small numbers of peptides will be

optimally effective as desensitisation therapies. The conclusion drawn in the art where MHC binding allergen epitopes have been identified is that even if an immunodominant epitope is identified, it would appear that it is required to react with a variety of restricted MHCs to be of therapeutic value (see Van Neerven RJJ *et al* (1994) *J Immunol* 152, 4203-4210; Higgins JA *et al* (1994) *J Allerg Clin Immunol* 93, 891-899).

As set forth herein, it has now been observed that a patient may be desensitised to a particular allergen by the administration of a peptide or a composition containing a peptide that is able to bind to at least one MHC molecule of said patient and which is able to induce a LPR in an individual who possesses the same MHC Class II molecule type. According to the present invention, therefore, the concept of "universal" desensitising peptides is rejected in favour of a selective approach which takes into account tissue type. Nevertheless, it will be appreciated that using a composition containing a plurality of peptides according to the present invention may be "universal" in the sense that a single composition may be used for most of the population, but that this is still selective on the basis that the composition contains peptides which are restricted by a particular MHC Class II molecule.

It can be hypothesised that eosinophil-dependent mucosal tissue damage, including LPR, is under T-cell control. For example, by *in situ* hybridisation the numbers of mRNA positive cells for the Th2-type (IL-4 and IL-5) and eosinophil-active cytokines (IL-3, IL-5 and GM-CSF) were shown to be elevated in asthmatics both at baseline (Robinson *et al* (1992)

*Am Rev Respir Dis* 146:1393-1400). Furthermore IL-4 and IL-5 mRNA co-localised largely to CD4<sup>+</sup> T cells (Ying *et al* (1997) *J Immunol* 158:3539-3544). A T cell component of the LAR is also

suggested by the observation that cyclosporin A attenuated the LAR, but not the EAR, provoked by allergen inhalation (Sihra *et al* (1997) *Thorax* 52:447-452). Furthermore a single infusion of anti-CD4 produced significant improvement in lung function in chronic corticosteroid-dependent asthmatics. However it has been difficult to determine whether T cell activation, as an initiating event, leads directly to airway narrowing in asthmatic patients and therefore an asthmatic response.

As described herein, it has now been shown that T cells can be selectively activated, and then rendered unresponsive. Moreover the anergising or elimination of these T-cells leads to desensitisation of the patient for a particular allergen. The desensitisation manifests itself as a reduction in response to an allergen or allergen-derived peptide, or preferably an elimination of such a response, on second and further administrations of the allergen or allergen-derived peptide. The second administration may be made after a suitable period of time has elapsed to allow desensitisation to occur; this is preferably any period between one day and several weeks. An interval of around two weeks is preferred.

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Based on these results, the invention provides a method for desensitising a patient to a polypeptide allergen which comprises the administration to the patient of a peptide specifically selected to induce LPR and subsequent desensitisation in the patient wherein the peptide is restricted by a particular MHC Class II molecule and capable of inducing LPR in an individual who possesses the given MHC Class II molecule. The peptide is selected according to whether they induce LPR

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LPR is defined as set forth in *Allergy and Allergic Disease*, 2nd edn, 1997, p. 100.

Kay (Ed.), Blackwell Science, pp 1113 to 1130, and includes asthmatic, cutaneous and nasal late phase responses as described above.

As noted above, the peptide which is administered may be included in a composition containing a plurality of peptides derived from the allergen.

Preferably, the peptides are derivatives of the allergen itself, and retain at least one common antigenic determinant of the allergen. "Common antigenic determinant" means that the derivative in question retains at least one antigenic function of the allergen. Antigenic functions include possession of an epitope or antigenic site that is capable of binding to TCRs which recognise the allergen or fragments thereof. Thus, the peptides provided by the present invention include splice variants encoded by mRNA generated by alternative splicing of a primary transcript encoding the allergen, amino acid mutants, glycosylation variants and other covalent derivatives of the allergen which retain at least an MHC-binding property of the allergen. Exemplary derivatives include molecules wherein the peptide of the invention is covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid. Further included are naturally occurring variants of the allergen found in a particular species. Such a variant may be encoded by a related gene of the same gene family, by an allelic variant of a particular gene, or represent an alternative splicing variant of the allergen gene.

25

Derivatives of the allergen also comprise mutants thereof, which may contain amino acid deletions, additions or substitutions.

Thus, conservative amino acid substitutions may be made to peptides according to the invention substantially without altering the nature of the

30



allergen, as may truncations from the N or C termini. Deletions and substitutions may moreover be made to the fragments of the allergen comprised by the invention. Peptides may be produced from a DNA which has been subjected to *in vitro* mutagenesis resulting eg in an addition,  
5 exchange and/or deletion of one or more amino acids. Preferably, peptides are produced by peptide synthesis according to known techniques using commercially available peptide synthesisers. Mutations and/or truncations may thus be made by changing the amino acid sequence during the synthesis procedure.

10

Suitable variants capable of binding to TCRs may be derived empirically or selected according to known criteria. Within a single peptide there are certain residues which contribute to binding within the MHC antigen binding groove and other residues which interact with hypervariable  
15 regions of the T cell receptor (Allen *et al* (1987) *Nature* 327:713-5). Within the residues contributing to T cell receptor interaction, a hierarchy has been demonstrated which pertains to dependency of T cell activation upon substitution of a given peptide residue. Using peptides which have had one or more T cell receptor contact residues substituted with a  
20 different amino acid, several groups have demonstrated profound effects upon the process of T cell activation. Evavold & Allen (1991) *Nature* 252:1308-10) demonstrated the dissociation of T cell proliferation and cytokine production. In this *in vitro* model, a T cell clone specific for residues 64-76 of haemoglobin (in the context of I-E<sup>k</sup>), was challenged  
25 with a peptide analogue in which a conservative substitution of aspartic acid for glutamic acid had been made. This substitution did not significantly interfere with T cell activation. However, when a T cell clone with this analogue, no proliferation was detected although IL-4 secretion was maintained, as was  
30 the capacity of the clone to help B cell responses. In a subsequent study

the same group demonstrated the separation of T cell-mediated cytotoxicity from cytokine production. In this instance, the former remained unaltered while the latter was impaired. The efficacy of altered peptide ligands *in vivo* was initially demonstrated in a murine model of EAE (experimental allergic encephalomyelitis) by McDevitt and colleagues (Smilek *et al* 5 (1991) *Proc Natl Acad Sci USA* 88:9633-9637). In this model EAE is induced by immunisation with the encephalitogenic peptide A<sub>1-11</sub> of MBP (myelin basic protein). Substitution at position four (lysine) with an alanine residue generated a peptide which bound well to its restricting 10 element (A $\alpha^u$ A $\beta^u$ ), but which was non-immunogenic in the susceptible PL/JxSJLF1 strain and which, furthermore prevented the onset of EAE when administered either before or after immunisation with the encephalitogenic peptide. Thus, residues can be identified in peptides which affect the ability of the peptides to induce various functions of 15 T-cells.

Advantageously, peptides may be designed to favour T-cell proliferation and induction of desensitisation. Metzler and Wraith have demonstrated improved tolerogenic capacity of peptides in which substitutions increasing 20 peptide-MHC affinity have been made (Metzler & Wraith (1993) *Int Immunol* 5:1159-65). The demonstration that an altered peptide ligand can cause long-term and profound anergy in cloned T cells (Sloan-Lancaster *et al* (1993) *Nature* 363:156-9) is particularly relevant to the applications of such peptide analogues in immunotherapy for diseases such as 25 autoimmunity and allergy, in addition to the induction of host/donor-specific tolerance in transplantation.

Furthermore, the fragments of the allergen comprise individual 30 domains thereof, as well as smaller polypeptides derived from the

domains. Preferably, smaller polypeptides derived from the allergen according to the invention define a single epitope of the allergen capable of binding a TCR. Fragments may in theory be almost any size, although smaller fragments are more likely to be restricted to a single MHC molecule and are thus preferred. Preferably, fragments will be between 5 and 50, preferably between 5 and 25, and advantageously about 17 amino acids in length. It is preferred if the peptides do not invoke an IgE response and do not lead to the release of histamine from enriched basophils or mast cell preparations from most sensitised individuals.

10

Candidate peptides potentially capable of inducing LPR in a patient may be preselected in order to maximise the chances of identifying a therapeutically useful peptide in *in vivo* tests. The steps of this aspect of the invention comprise the determination that the peptide is MHC Class II restricted, for example it is capable of causing T-cell proliferation when associated with an MHC molecule present in the patient to be treated. Thus, in a particular embodiment the selection procedure can be broken down into three steps, performed either sequentially (in any order) or together:

20

- a) assaying a peptide or peptides in a T-cell activation assay and selecting peptides capable of inducing activation in an individual's T-cells;
- b) tissue-typing the individual to determine MHC Class II type; and
- c) determining the MHC Class II molecule bound by each candidate peptide.

25

Steps (a) and (c) in particular, may be combined in a single T-cell activation assay. Preferably, the assay involves the use of cells transfected

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to express a particular MHC molecule, and the binding of the peptide to this MHC assessed by its ability to induce T-cell proliferation in the presence of the transfected cells alone. Suitable transfected cells are readily available and can, in any case, be readily made by transfecting the  
5 cloned genes into suitable cell lines.

Preferably, a peptide selected according to the above procedure is tested for its ability to induce LPR in an individual. If LPR is induced, repeated administration will result in desensitisation to the allergen from which the  
10 peptide is derived.

However, once a peptide has been determined to bind a particular MHC Class II type and to be capable of inducing LPR when administered to an individual possessing that MHC Class II type, it can be used to induce  
15 desensitisation to the relevant allergen in substantially any patient possessing the required MHC Class II molecule. Therefore, peptides derived from particular allergens may be characterised according to their binding to particular MHC Class II types and their ability to induce LPR, thus providing a database from which a suitable peptide may be selected  
20 for any given patient upon tissue typing of that patient. Additionally or alternatively, a preparation containing a plurality of MHC-binding peptides capable of inducing LPR may be employed which will be effective in desensitising the majority of sensitised individuals.

25 Thus, in one embodiment antigen presenting cells may be isolated from a patient known to be sensitive to a particular allergen or allergens, and based on the peptide-binding MHC molecule.

30 The invention accordingly provides a method for selecting a peptide for use as an immunotherapeutic

agent for desensitising a patient to an allergen comprising the steps of:

- a) tissue-typing the patient to determine MHC Class II type; and
- 5 b) selecting, from a database of peptides which are known to bind to particular MHC Class II molecules and induce a late phase response in an individual possessing such MHC Class II molecules, one or more peptides capable of binding to the MHC Class II molecules possessed by the patient.

10

For the avoidance of doubt, the individual referred to in part (b) above need not necessarily be the same individual as the patient undergoing treatment whom is tissue typed in part (a). In fact, once the MHC Class II restriction of a particular allergen-derived peptide is determined, and it has  
15 been determined that the peptide is capable of inducing a LPR in an individual, particularly an appropriately sensitised individual, who possesses the said MHC Class II molecule, there is no requirement to test the ability of the patient's own MHC Class II molecules.

- 20 Allergens that may be amenable to desensitisation procedures as described herein include the peptides derived or chosen from the list comprising the allergens; Fel d 1 (the feline skin and salivary gland allergen of the domestic cat *Felis domesticus* - the amino acid sequence of which is disclosed in WO 91/06571), Der p I, Der p II, Der fI or Der fII (the  
25 major protein allergens from the house dust mite dermatophagoides - amino acid sequences disclosed in WO 94/24281).

30 Allergens that may be amenable to desensitisation procedures as described herein include the peptides derived or chosen from the list comprising the allergens present in any of the following: grass, tree and weed (including ragweed) pollens; fungi and moulds; foods eg fish, shellfish, crab lobster

peanuts, nuts, wheat gluten, eggs and milk; stinging insects eg bee, wasp and hornet and the chironomidae (non-biting midges); spiders and mites, including the house dust mite; allergens found in the dander, urine, saliva, blood or other bodily fluid of mammals such as cat, dog, cows, pigs, sheep, horse, rabbit, rat, guinea pig, mouse and gerbil; airborne particulates in general; latex; and protein detergent additives.

Where the allergen is an insect protein, the peptides may be selected from the group comprising: housefly, fruit fly, sheep blow fly, screw worm fly, grain weevil, silkworm, honeybee, non-biting midge larvae, bee moth larvae, mealworm, cockroach and larvae of *Tenibrio molitor* beetle. All these being insect allergens, they are of particular relevance to allergic problems arising in the workplace.

15 Where the allergen is the Fel d 1 allergen, useful peptides may preferably comprise a sequence as shown in any one of SEQ ID Nos. 1 to 3. Particular preferred peptides for use in the methods of the invention are those with the sequence given in SEQ ID Nos. 1 or 2 or 3. Preferred compositions of the invention include those that contain the peptides with  
20 the sequence given in SEQ ID Nos. 1, 2 and 3, and compositions containing the MHC Class II-restricted peptides of the thirteen peptides described in Example 7 and for which can be determined a LPR in an individual possessing appropriate MHC Class II molecules.

A database according to the invention includes information on the MHC Class II molecule(s) bound by peptides in the database and the ability of the peptides to induce a LPR in patients possessing said MHC Class II molecule(s). The database may also include information on whether or not a particular peptide is potentially capable of eliciting a LPR and therefore desensitisation in a particular patient on the basis of that patient's tissue

type.

The invention moreover provides a peptide listed in a database according to the invention, for use in therapy. Preferably, such peptides are useful  
5 in methods for desensitising patients to allergens in accordance with the methods set forth herein. Peptides to be included in the database, and peptides which may be useful either individually or as a mixture in a composition of the invention may readily be selected by the methods of the invention from polypeptide allergens whose polypeptide sequences, or  
10 reference to polypeptide sequences, are given in Example 6.

The MHC molecules expressed on APCs which bind peptides derived from a specific allergen may be identified by methods known in the art, such as T cell proliferation studies with MHC blocking antibodies, and  
15 PCR techniques, for example techniques based on those of Olerup & Zetterquist (1992) *Tissue Antigens* 29:225-235. Thus, antigen-presenting cells, expressing a variety of MHC molecules may be incubated with allergen and T cells and the latter observed for proliferation. Addition of antibodies to specific MHC classes may then be made in repeat  
20 incubations in order to identify the restricted MHC in respect of the allergen being tested. See Van Neerven RJJ *et al* (1994) *Immunol* 82:351-356, and Yssel H *et al* (1992) *J Immunol* 148:738-745.

Alternatively, cells presenting a single MHC Class II type, for example  
25 cells such as fibroblast cells transfected with the genes encoding an MHC Class II molecule, may be incubated with individual peptides for which the appropriate MHC Class II molecule is known. Incubation of a particular peptide presented by the appropriate MHC Class II molecule will lead to T-cell proliferation. T cell proliferation is not the  
30 only indicator that a particular peptide binds to a particular MHC Class II

molecule on an APC. Other indicators include the secretion of measurable soluble products such as cytokines, changes in intracellular calcium levels, and other means of measuring T cell activation which are well known in the art.

5

Preferred fibroblasts for use in this aspect of the invention include human or murine fibroblasts, particularly L-cells.

The latter method may be used in a combinatorial approach, in which  
10 groups of peptides may be tested together and effective peptides identified by standard combinatorial techniques.

Specific epitopes of the allergen or peptide derived therefrom that bind to at least one MHC Class II molecule may then be identified by standard  
15 procedures and used in desensitisation procedures as described herein. Accordingly, the invention provides peptides when selected according to the foregoing aspects of the invention.

For example, when the allergen is a cat allergen such as the Fel d 1  
20 protein, then the MHC molecule may include DR13 or DR1 class II MHC, and a peptide that binds to DR13 and/or DR1 or any of its sub-types that may be used in a desensitisation procedure is that shown in SEQ. ID No. 3.

25 The peptides identified in such a manner, and those of use in the methods of the present invention may be used in desensitisation procedures that typically involve sequential administration.

As has been described elsewhere the peptide or composition  
30 administered to the patient may be at a concentration that does not invoke



a measurable or observable LPR. Subsequent administration will lead to desensitisation of the patient. For example, if the peptide is that of SEQ. ID No. 3 (a fragment of the Fel d 1 allergen), then upon first administration of this peptide a LPR will be observed. Subsequent  
5 administration of this peptide results in a weaker reaction or no reaction, the patient having been desensitised.

The invention also relates to the use of a peptide in desensitising a patient against an allergen, the peptide being identified by its capability to bind to  
10 at least one MHC Class II molecule present in an individual and induce LPR in an individual who possesses the said MHC Class II molecule, wherein the patient also possesses the given MHC Class II molecule.

Peptides may be administered to a patient singly or in combination (for  
15 example as a composition as defined above). Thus, the database according to the invention may be used to prepare a designer vaccine which may be used to desensitise a patient to a chosen allergen, on the basis of the patient's MHC Class II type. The MHC Class II type can be correlated to the known MHC Class II binding characteristics of the  
20 peptides listed in the database, and the appropriate peptides selected and combined to form a designer vaccine. Similarly, the database may be used to design compositions (ie mixtures of peptides) which contain sufficient number of peptides, each of which demonstrate restriction to a particular MHC Class II molecule and which are able to induce a late  
25 phase response in an individual who possesses the said MHC Class II molecule, such that for at least 75% (preferably at least 80% or 85% or 90%) of the population.

The database according to the invention is capable of inducing a late phase response in an individual with the appropriate MHC Class II molecule.

- Whilst it may be possible to design a vaccine which targets all or most of the epitopes on a particular antigen, this is unnecessary due to linked suppression of T-cells. Linked suppression is a phenomenon in which administration of a single epitope from a protein leads to the induction of a population of regulatory peptide-specific T lymphocytes which, by release of soluble factors such as TGF $\beta$  and/or IL-10, are able to suppress or modify responses of non-tolerant T cells specific for other epitopes within the same protein and in some models epitopes derived from other proteins ("bystander suppression") (Davies *et al* (1996) *J Immunol* 156:3602-7).
- 10 In transplantation models, such regulatory T cells have been demonstrated to be capable of inducing a similar phenotype in naive T cells. This has given rise to the term "infectious tolerance" (Qin *et al* (1993) *Science* 259:974-7) which may be a mechanism for effecting long-term hyporesponsiveness.
- 15 Linked suppression is thought to occur when peptide-specific regulatory T cells engage peptide/MHC complexes on the surface of the same or neighbouring APC as T cells specific for other epitopes. The latter may be responding to epitopes derived from the same molecule as the regulatory T cells or from a distinct molecule being processed by the same APC. This phenomenon allows desensitisation of patients to one or multiple allergens by the administration of a limited number of peptides.
- 20

Whilst it may be possible for the peptides or compositions according to the invention to be presented in raw form, it is preferable to present them as a pharmaceutical formulation. Thus, according to a further aspect, the present invention provides a pharmaceutical formulation comprising:  
peptide(s) or peptide composition(s);  
one or more pharmaceutically acceptable carriers, adjuvant(s) and optionally one or more other therapeutic ingredients. The carrier(s) must be 'acceptable' in

25

the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. Typically, carriers for injection, and the final formulation, are sterile and pyrogen free.

- 5 The formulations include those suitable for oral (particularly inhaled), parenteral (including subcutaneous, transdermal, intradermal, intramuscular and intravenous and rectal) administration, although the most suitable route may depend upon for example the condition and disorder of the recipient. The formulations may conveniently be presented
- 10 in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association a compound of the present invention as herein defined or a pharmacologically acceptable salt or solvate thereof ("active ingredient") with the carrier which constitutes one or more accessory ingredients.

15

- Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a
- 20 non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Formulations for inhalation may be presented in any of the ways known to be effective eg metered dose inhalers.

- 25 Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and chelating agents, and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose
- 30

containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example, water-for-injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol.

10

Preferred unit dosage formulations are those containing an effective dose, as hereinbelow recited, or an appropriate fraction thereof, of the active ingredient.

15 The compounds of the invention may typically be administered intranasally, by inhalation, orally or *via* injection at a dose of from 0.0001 to 1 µg/kg per dose. Preferred are doses in the region of 10 to 150 µg per human patient, advantageously about 80 µg.

20 A further aspect of the invention provides a method of determining an initial dose of an immunotherapeutic peptide for desensitising a patient to a polypeptide allergen, which peptide is derived from the allergen and wherein restriction to a MHC Class II molecule possessed by the patient can be demonstrated for the peptide and the peptide is able to induce a late phase response in an individual who possesses the said MHC molecule, the method comprising (1) determining the dose which is able to generate an observable late phase response in a given proportion of individuals who  
late phase response and (2) selecting a lower dose which is incapable of  
30 inducing an observable late phase response in substantially all individuals

who possess the said MHC molecule and in whom the peptide is able to induce a late phase response.

Preferably, the individuals who possess the said MHC molecules are  
5 appropriately sensitised; that is to say that the individuals have been sensitised previously to the allergen in question.

The initial dose which is administered to the patient to be desensitised is, as is described above, one which may not itself give rise to an observable  
10 LPR.

In step (1) of the method of determining an initial dose the given proportion of individuals may be any suitable proportion of, but not all, individuals as given. Typically, the proportion is 50% of individuals as  
15 given, but it may be, for example, 30% or 40% or 60% or 70% of individuals as given. In step (2), the lower dose may be the maximum dose that is incapable of inducing an observable late phase response in substantially all individuals who possess the said MHC molecules and in whom the peptide is able to induce a LPR.

20

Typically, but it will be appreciated that this will vary from peptide to peptide, the lower dose is between 10-fold and 100-fold lower than the dose which induces an observable LPR in 50% of suitable individuals (a  
25 suitable individual is one who is appropriately sensitised and has the appropriate MHC Class II molecule(s) to facilitate peptide reactivity.

Immediate reactions are determined in asthmatics, late nasal reactions in rhinitis and late phase skin reactions in all allergic individuals.

30

It is preferred if the LPR is a late cutaneous reaction.

The methods of the invention are particularly suited for use in connection with human patients. However, it will be appreciated that animals, particularly mammals, and more particularly domestic and farm animals such as dogs and cats, may suffer from allergies due to polypeptide allergens. The methods of the invention include methods in connection with such animals. Although the specification refers to MHC and HLA Class II molecules, equivalent molecules exist in mammals other than humans as is well known in the art.

The invention is further described, for the purpose of illustration only, in the following examples, which refer to the figures.

Figure 1. The three peptides comprising FC1P (solid circles; 80µg) or vehicle control (open circles) are injected intradermally at time zero on two separate days. Forced expiratory volume in 1 second (FEV1) is measured at intervals as a readout of lung function over a 24hr period. The use of rescue medication is indicated by arrows.

Figure 2. Repeated administration of FC1P leads to a reduced lung response. Three patient volunteers who develop a late asthmatic reaction following administration of FC1P (closed circles), are challenged again with the same dose after a period of at least 2 weeks. No significant fall in FEV1 is observed following the second challenge (closed triangles). Open circles indicate the control day. Arrows indicate the use of bronchodilators.

Figure 3. Two DRB1\*1301 and DRB1\*1302 variants, DRB1\*1301 and DRB1\*1302 are incubated overnight with each of the three FC1P peptides, or a

control peptide, or medium alone. Cells are washed and incubated for one hour with a cytostatic agent to prevent proliferation in the subsequent assay. L cells are then incubated for 48 hours with T cells from a T cell line raised to whole cat dander (and including the Fel d 1 protein).

5 Proliferation of the T cells is measured by their incorporation of the radiolabelled compound  $^3\text{H}$ -thymidine. T cells demonstrate a statistically significant response to the DR13 L cells and peptide FC1P3 (KALPVVLENARILNCV) but not to the other peptides/control.

10 Figure 4. Human fibroblasts expressing the DR1 allele DRB1\*0101 are incubated overnight with each of the three FC1P peptides, or medium alone, as described for Figure 3. In T cell proliferation assays, T cells demonstrate a statistically significant response to the DR1 expressing cells and peptide FC1P3 (KALPVVLENARILNCV) but not to the other

15 peptides/control.

Figure 5. Human fibroblasts expressing the DR4 alleles DRB1\*0404 and DRB1\*0405 are incubated overnight with each of the three FC1P peptides, or medium alone, as described for Figure 3. Figure 5 a) and b):

20 in T cell proliferation assays, DRB1\* 0408 responder cells demonstrate a statistically significant response to the DRB1\*0405 expressing cells and peptide FC1P2 (EQVAQYKALPVVLENA) but not to DRB1\* 0404 expressing cells and peptide FC1P2 or to the other peptides/control.

25 Figure 6. Human fibroblasts expressing the DR4 allele DRB1\*0405 are incubated overnight with each of the three FC1P peptides, or medium alone, as described for Figure 3. In T cell proliferation assays, DRB1\* 0408 responder cells demonstrate a statistically significant response to the DRB1\*0405 expressing cells and peptide FC1P2 (EQVAQYKALPVVLENA) but not to the other peptides/control.

30

Figure 7. The T cell proliferation responses observed in Figures 3, 4 and 6 are confirmed by [IL-5] measurement in Figures 7 (a), 7(b) and 7 (c) respectively. As expected, these results show that IL-5 production correlates with T-cell proliferation.

Figure 8. Hypothetical protein and peptides (15mers) derived from overlapping by one residue.

Figure 9. Multiple overlapping peptides (MOP) from the cat allergen Fel d I. The three sequences within the box were insoluble in aqueous solution and as a result were excluded from the MOP preparation for clinical use.

Figure 10. An example of a LAR induced by the Fel d I MOP. The intradermal administration of 13 peptides which comprise MOP (solid circles; 2.5 µg, day 1) induce a fall in FEV1 of greater than 20% at 3 hours. Control day administration of 30BU cat dander extract does not induce a fall in FEV1 (open circles). A second administration of MOP (solid triangles; 2.5 µg, day 66) results in an attenuated fall in FEV1 which does not reach 20%. Arrows indicate the use of rescue medication ( $\beta_2$  agonists).

Figure 11. Changes in the cutaneous late phase response to whole allergen 6 hours after intradermal administration of whole cat dander extract before and after intradermal administration of MOP.

Figure 12. The effect of intradermal administration of MOP on the late phase response to cat allergen administered intradermally to cat allergic asthmatic subjects inducing a fall in FEV1 of greater than 20% compared



to a control day (open circles; 30BU whole cat dander extract, Figures 12(a), (b) and (c)). A second administration of FC1P within 6 weeks (closed down triangles; 80  $\mu$ g, Figure 12(a)) demonstrated an attenuation of the response. Following administration of FC1P greater than one year after the initial dose (closed up triangles; 80  $\mu$ g, Figures 12(a), (b) and (c)), a fall in FEV1 of similar magnitude to the initial injection was observed. Arrows indicate the use of rescue medication ( $\beta_2$  agonists).

*Schedule of sequences for sequence listing:*

- 10 SEQ ID No 1: LFLTGTPDEYVEQVAQY (FC1P1)  
SEQ ID No 2: EQVAQYKALPVVLENA (FC1P2)  
SEQ ID No 3: KALPVVLENARILKNCV (FC1P3)  
SEQ ID No 4: Fel d I chain 1 in Figure 9  
SEQ ID No 5: Fel d 2 chain 2 in Figure 9

15

Other SEQ ID Nos. for peptides are shown on Figure 9.

**EXAMPLES**

20 **Experimental Techniques**

*Primary Proliferation Assays*

PBMCs are separated from whole blood by density gradient centrifugation according to standard methods. Cultures are established at  $2 \times 10^5$  cells per well in flat bottomed 96 well plates with 3 concentrations each individual peptide, or an optimum concentration of cat dander cat allergen extract, medium (negative control) or PPD (positive control). Cells are cultured for 8 days.

Cells are counted after 8-16 hours

### *T Cell Clones*

PBMCs are cultured in 24 well plates with cat dander for 10 - 12 days, with the addition of approximately 10ng IL-2 on days 5 and 7, restimulated twice with irradiated autologous PBMCs and cat dander, and  
5 the line expanded with Phytohaemagglutinin (PHA) and IL-2. Clones are established by limiting dilution and will subsequently be frozen for use at a later stage to determine changes in cytokine secretion.

### Example 1: Preparation Of Allergen Peptides

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The sequence of chain 1 of the cat allergen Fel d 1 is shown in Figure 9 (SEQ. ID. No. 4); chain 2 is also shown in Figure 9 (SEQ. ID. No. 5). Multiple overlapping peptides are designed around this sequence, as well as that of chain 2 of Fel d 1, as shown in Figure 9.

15

### Example 2: Observation Of LAR In Patients On Peptide Administration

A single intradermal administration (80µg of each peptide) of a mixture  
20 containing three short peptides (Figure 9; (SEQ. ID Nos. 1, 2 or 3)) is given to 18 cat asthmatic individuals. 6 patients develop an isolated late asthmatic reaction as shown in Figure 1 wherein a greater than 20% fall in Forced Expiratory Volume in 1 second (FEV1 - a measure of lung function) is considered as a positive asthmatic effect. The results are  
25 shown in Figure 1 where the three peptides comprising FC1P [solid circles; FC1P comprises FC1P1 (SEQ. ID. No. 1), FC1P2 (SEQ. ID. No. 2) and FC1P3 (SEQ. ID. No. 3)] or vehicle control (open circles) are injected intradermally.

Rescue medication is given if necessary over a 24hr period. The use of rescue medication is indicated by arrows.

This result demonstrates that peptides capable of causing a LPR can be derived from a common allergen such as cat dander and tested for LAR production in cat asthmatic individuals.

5

Three patient volunteers who develop a late asthmatic reaction following administration of FC1P (closed circles), are challenged again with the same dose after a period of at least 2 weeks. No significant fall in FEV1 is observed following the second challenge (closed triangles). Open circles indicate the control day. Arrows indicate the use of bronchodilators. As shown in Figure 2, none of the three develop a late asthmatic reaction to the second peptide administration indicating that the immune response to this peptide has been downregulated.

### 15 Example 3: Correlation between Tissue type and LAR

The 18 patients observed in Example 2 are MHC-typed using PCR, based upon the method of Olerup & Zetterquist (1992) *Tissue Antigens* 29:225-235. Four of the 6 reactors express HLA-DR13 (a closely related family of MHC molecules) compared to 1 out of 12 of the non-reactors. These results indicate that one of the three peptides injected is capable of binding to a DR13 family member and thus stimulating peptide-specific T cells from the reactors.

25 In order to demonstrate that specific T cells have been activated, L cells which have been transfected with the human genes encoding DR13

are used. Murine T cells expressing two DR13 variants, DRB1\*1301 and 1302 are incubated overnight with each of the three FC1P peptides, or a control peptide, or medium alone. Cell

are washed and incubated for one hour with a cytostatic agent to prevent proliferation in the subsequent assay. L cells are then incubated for 48 hours with T cells from a T cell line raised from PBMCs isolated from a reactor patient as described above and stimulated weekly with whole cat dander (and including the Fel d 1 protein). Proliferation of the T cells is measured by their incorporation of the radiolabelled compound <sup>3</sup>H-thymidine. T cells demonstrate a statistically significant response to the DR13 L cells and peptide FC1P3 (SEQ. ID No 3) but not to the other peptides/control as shown in Figure 3.

10

A further experiment is performed with human fibroblasts expressing the DR1 variant DRB1\*0101. Cells are incubated overnight with each of the three FC1P peptides, or medium alone, washed, treated and incubated with T-cells as described above for the DR13 variants. T cells demonstrate a statistically significant response to the DR1 L cells and peptide FC1P3 (SEQ. ID No 3) but not to the other peptides/control as shown in Figure 4.

It is demonstrated that FC1P3 is capable of binding to both DR1 and DR13 MHC molecules and activating T cells, thereby inducing the isolated late asthmatic reaction shown in Figure 1. This result correlates extremely well with the tissue type data obtained from the patient population, wherein 4 out of six reactors are DR13 and two are DR1, compared with 1 out of 12 DR1 and 1 out of 12 DR13 non-reactors.

25

In a further series of experiments, patients reacting to FC1P are identified which express HLA DR1 (DRB1\*0101) and DR13 (DRB1\*0103). The results are shown in Figure 5 and Figure 6.

The results indicate that patients expressing DRB1\* 0408 respond to FC1P2 presented by 0405 L cells but not 0404 L cells or to other peptides or controls. Likewise, patients expressing DRB1\* 0405 respond to FC1P2 presented by 0405 L cells but not to other peptides or controls.

Figure 7 shows the IL-5 secretion levels for DR13(a), DR1(b) and DR4(c) HLA types which correlate with T cell proliferation data as expected.

Example 4: FC1P3 induces LAR and desensitisation in tissue-typed patients

Patients are selected on the basis of being allergic to cat dander, as in the previous examples. T-cell lines are prepared from each patient as described above, and maintained with weekly stimulation with cat dander extract. The patients are tissue-typed, and patients possessing DR1 or DR13 variants selected.

In order to predict the ability of peptide FC1P3 to desensitise the patients against cat dander, T-cell proliferation assays are performed using T-cells isolated from the patients as described and human fibroblasts or murine L-cells transfected with DR1 or DR13 alleles in the presence of FC1P3 according to Example 3. The T-cells are observed to proliferate, by the incorporation of <sup>3</sup>H-thymidine, indicating that T-cells isolated from DR13 and DR1 possessing patients are responsive to stimulation with the FC1P3 peptide.

These patients are then challenged with cat dander, and are found to be positive and in respect of whom a positive result has been obtained in the T-cell proliferation assay. These patients experience a LAR response, as

measured by a 20% or greater fall in FEV<sub>1</sub>.

Patients who develop a late asthmatic reaction following administration of FC1P3 are challenged again with the same dose after a period of 2 weeks.

- 5 As in Example 2, no significant fall or a reduced fall in FEV<sub>1</sub> is observed following the second challenge, indicating that the immune response to this peptide has been downregulated.

#### Example 5: MHC restriction mapping of Fel d 1

10

In order to prepare a database of Fel d 1 derived peptides characterised according to MHC type restriction, an *in vitro* study of MHC class II restriction mapping is performed using a panel of L cells, T-cell lines to whole cat allergen and the overlapping peptides from chain 1 and chain 2,  
15 as described in Example 1. T cell lines with specificity for whole cat extract (which includes Fel d 1) are generated from the peripheral blood of subjects before peptide administration according to the procedures described above. Subjects are HLA-DR, DP and DQ typed, and, based on their expression, initially of DR alleles, transfected fibroblasts are  
20 selected to assay T-cell stimulation by each of the peptides.

Where the required HLA type clone is not available, MHC genes are cloned directly from the patient's cells by PCR amplification and cloning, as described above. Cloned genes are subsequently expressed in murine  
25 L-cells.

Cell lines (generous gifts from Prof. J.R. Lamb, University of Edinburgh, Prof. R.I. Lechler and Dr. G. J. Goldstein, University of California, San Francisco, USA) expressing the appropriate restriction element

are incubated with each individual Fel d 1 peptide as described in Example 3. Equivalent cell lines are generally available or may be readily made by transfecting appropriate genes expressing MHC Class II molecules. Following incubation in the cytostatic agent mitomycin C to prevent L cell division, cells are extensively washed and incubated with the T cell line. Proliferative responses are measured after 48 hours by addition of tritiated thymidine to all cultures for 8-16 hours. Peptides eliciting a proliferative response from the T cell line are thus restricted by the HLA allele expressed by the chosen L cell line.

10

Administration of peptides obtained from the database to patients possessing the HLA type in respect of which a proliferative response is seen in the above assay in the majority of cases results in a LAR, as expected, which is followed by desensitisation of the patient to cat dander on subsequent administration of the peptides.

In this way an MHC class II restriction map of the Fel d 1 molecule is constructed such that the appropriate peptides for immunotherapy may subsequently be selected on an individual patient basis, solely by virtue of that subject's HLA type.

20

Example 6: Identification of MHC-restricted peptides capable of inducing late phase reactions in individuals possessing the appropriate MHC molecule

25

(1) Overlapping peptides of 15 amino acid residues (range approx. 7-20) which are offset by one residue are chemically synthesised, an example being  $\text{Phe-Asp-Val-Tyr-Ile-His-Pro-Phe-Phe-Val-Tyr-Ile-His-Pro-Phe}$  (sequence 100-114 of the Fel d 1 molecule). An example dimer (100-114) which may be derived from it is given in figure 8.

- (2) Each individual peptide is incubated with murine or human cells such as fibroblasts for example, which have been transfected with, or  
5 already express, the genes encoding a particular MHC molecule such as, for example DRA and DRB1\*0101. The concentration of peptide used for the incubation stage may vary from approximately 0.01mg/ml to 1mg/ml or more. An example is 200µg/ml. The incubation period may vary from approximately a few minutes to several hours. An example is 16 hours.
- 10 (3) Following incubation with peptide, the cells are washed several times (for example 3 times) in tissue culture medium (for example RPMI-1640 medium supplemented with 5% normal human AB serum, 2mM L-glutamine, 100microgram/ml streptomycin and 100U/ml penicillin).
- 15 (4) Cells are then incubated with mitomycin C (at approximately 50µg/ml) or another suitable cytostatic agent to prevent cell division. Cells are washed several times (for example 5 times) in culture medium and dispensed into 96 well tissue culture plates at a concentration of  
20 approximately  $3 \times 10^4$  cells per well for example.
- (5) To these cells are added approximately  $1 \times 10^4$  cells of a T lymphocyte cell line which has been cultured in the presence of, and is reactive with, the protein from which the peptides in step (1) were  
25 derived. The MHC molecules expressed by the individual from which the T lymphocyte line was raised would usually include the MHC molecule expressed on the cells in step (2). Alternatively, the MHC molecule expressed by the individual from which the peptides in step (1) were derived is added to the cells in step (2). Additionally, T lymphocytes from the same cell line are cultured on their own and also



with the MHC-expressing cells described in stage (2) which have either not been incubated with a peptide, or have been incubated with an irrelevant peptide such as a peptide from another protein.

5 6) The cell mixture is cultured for approximately 2-3 days prior to the addition to each well of approximately 37MBq (1 $\mu$ Ci) of tritiated thymidine or similar for several hours (for example 6-16 hours).

7) Cultures are then harvested onto glass fibre filters and cellular  
10 proliferation (of the T lymphocytes), as correlated with uptake of tritiated thymidine into the DNA of the cells, is measured by liquid scintillation spectroscopy or a similar technique.

Peptides capable of binding to the relevant MHC molecules and inducing  
15 T cell activation are identified by the incorporation of the tritiated thymidine into the newly synthesised DNA of the activated T cells. When the DNA is analysed by liquid scintillation spectroscopy (or other suitable techniques) the radioactive label (tritium) generated counts per minute which correlate with the degree of T cell proliferation and thus activation.

20

Thus, MOPs derived from a polypeptide allergen are useful principally in the selection procedure for identifying the one or more useful peptides (which show MHC Class II restriction and which are able to give rise to a LPR in an individual who possesses the appropriate MHC Class II  
25 molecules) which may be used either individually or in combination as an immunotherapeutic agent.

The following is a list of known allergen sequences and database accession numbers (NCBI Entrez accession numbers). NCBI is the National Center  
30 for Biotechnology Information and is available at <http://www.ncbi.nlm.nih.gov/>.

→ 200  
Ch  
Institutes of Health. The NCBI web site, from which access to the database may be sought, [www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/). The allergens may be used as described above in order to identify MHC-restricted peptides capable of inducing LPR in individuals who possess a particular MHC molecule.

Allergen sequences and database accession numbers (NCBI Entrez accession numbers):

10 **House dust mite**

*Dermatophagoides pteronyssinus*

Der p 1

MKIVLAIASLLALSAVYARPSSIKTFFEEYKKA FNKSYATFEDEEAAR  
15 KNFLESVKYVQSNGGAINHLSDSLDEFKNRFLMSAEAFEHLKTQF  
DLNAETNACSINGNAPAEIDLRQMRTVTPIRMQGGCGSCWAFSGV  
AATESAYLAYRNQSLDLAEQELVDCASQHGCHGDTIPRGIEYIQHN  
GVVQESYYRYVAREQSCRRPNAQRFGISNYCQIYPPNVNKIREALA  
QTHSAIAVIIGIKDLDAFRHYDGRTIIQRDNGYQPNYHAVNIVGYSN  
20 AQGVDYWIVRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVIL

Der p 2

MMYKILCLSLLVA AVARDQVDVKDCANHEIKKVLVPGCHGSEPCII  
HRGKPFQLEAVFEANQNTKTAKIEIKASIDGLEVDVPGIDPNACHY  
25 MKCPLVKGQQYDIKYTWNVPKIA PKSENVVVTVKVMGDDGVLAC  
AIATHAKIRD

SSHFCCGGTILDEYWILTA AHCVAGQ TASKLSIRYNSLKHSLGGEKIS

206  
23  
1  
VAKIFAHEKYDSYQIDNDIALIKLKSPMKLNQKNAKAVGLPAKGSD  
VKVGDQVRVSGWGYLEEGSYSLPSELRRVDIAVVS RKECNELYSKA  
NAEVTDNMICGGDVANGGKDSCQGDSSGPPVVDVKNNQVVGIVSW  
GYGCARKGYPGVYTRVGNFIDWIESKRSQ

5

Der p 4

KXNPHFIGXRSVITXLME

Der p 5

10 MKFIIAFFVATLAVMTVSGEDKKHDYQNEFDL LMERIHEQIKKGE  
LALFY LQE QINHFE EKPTKEMKDKIVAEMDTI IAMIDGVRGVLDRL  
MQRKDL DIFEQYNLEMAKKSGDILERDLKKEEARVKKIEV

Der p 6

15 AIGXQPAAEAEAPFQISLMK

Der p 7

MMKLL LIAAAAFVAVSADPIHYDKITEEINKAVDEAVAAIEKSETFD  
PMKVDPDHSDKFERHIGIIDLKGELDMRNIQVRGLKQMKRVGDANV  
20 KSEDGVVKAHLLVGVHDDVVSMEYDLAYKLGD LHPNTHVISDIQD  
FVVELSLEVSEEGNMTLTSFEVRQFANVVNHIGGLSILDPIFAVLSD  
VLTAIFQDTVRAEMTKVLAPAFKKELERNNQ

Der p9

25 IVGGSNASPGDAVYQIAL

Dermatophagoides farinosus

Der 1

30 MKFVLAIASLLVLT VYARPASIKTFEFKKAFNKNYATVEEEEVARK

5 HTAIAVIIGIKDLRAFQHYDGRTHQHDNGYQPNYHAVNIVGYGSTQ  
GDDYWIVRNSWDTTWGDSGYGYFQAGNNLMMIEQYPYVVIM

10 MISKILCLSLLVAAVVADQVDVKDCANNEIKKVMVDGCHGSDPCII  
HRGKPF<sup>-</sup>TLEALFDANQNTKTAKIEIKASLDGLEIDVPGIDTNACHFM  
KCPLVKGQQYDIKYTWNVPKIAPKSENVVVTVKLIGDNGVLACAIA  
THGKIRD

15 MMILTIVLLAANILATPILPSSPNATIVGGVKAQAGDCPYQISLQSS  
SHFCGGSILDEYWILTAAHCVNGQSAKKLSIRYNTLKHASGGEKIQV  
AEIYQHENVDSMTIDNDVALIKLKTPTMTLDQTNAPVPLPAQGSVD  
KVGDKIRVSGWGYLQEGSYSLPSELQRVDIDVVSREQCDQLYSKAG  
ADVSENMICGGDVANGGVDSCQGDSGGPVVDVATKQIVGIVSWGYY  
20 GCARKGYPGVYTRVGNFVDWIESKRSQ

AVGGQDADLAEAPFQISLLK

MMKFLLIAAVAFVAVSADPIHYDKITEEINKAIDDAIAAIEQSETIDP  
MKVPDHADKFERHVGIVDFKGE LAMRNIEARG LKQMKRQGDANV  
KGE EGIVKAHLLIGVHDDIVSMENDL

[illegible]

*vt* Additional mite allergen sequences (NCBI entrez accession):

1170095; 1359436; 2440053; 666007; 487661; 1545803; 84702; 84699;  
5 625532; 404370; 1091577; 1460058; 7413; 9072; 387592.

### Cat

#### Felis sequences

10 1082946 Fel dI chain 2 precursor - cat

MRGALLVLALLVTQALGVKMAETCPIFYDVFFAVANGNELLLDLS  
LTKVNATEPERTAMKKIQDCYVENGLISRVLDGLVMTTISSSKDCM  
GEAVQNTVEDLKLNTLGR

15 1082945 Fel dI chain 1 short form - cat

MLDAALPPCPTVAATADCEICPAVKRDVDLFLTGTGPDEYVEQVAQ  
YKALPVLLENARILKNCVDAKMTEEDKENALSLLDKIYTSPLC

1082944 Fel dI chain 1 long form precursor - cat

20 MKGARVLVLLWAALLLIWGGNCEICPAVKRDVDLFLTGTGPDEYVE  
QVAQYKALPVLLENARILKNCVDAKMTEEDKENALSLLDKIYTSPL  
C

Additional Felis sequences (NCBI entrez accession):

25

539716; 539715; 423193; 423192; 423191; 423190; 1364213; 1364212;  
395407; 163827; 163828; 163829

### Latex

30 Hevea sequences:

## Hev b 1

MAEDEDNQQGQGEGLKYLGFVQDAATYAVTTFSNVYLFKDKSG  
 PLQPGVDIIEGPVKNVAVPLYNRFSYIPNGALKFVDSTVVASVTIHDR  
 5 SLPPIVKDASIQVVSAIRAAPEAARSLASSLPGQTKILAKVIFYGEN

## Hev b 3

MAEEVEEERLKYLDVRAAGVYAVDSFSTLYLYAKDISGPLKPGV  
 DTIENVVKTVPVYYIPLEAVKFVDKTVDVSVTSLDGVVPPVIKQ  
 10 VSAQTYSVAQDAPRIVLDVASSVFNTGVQEGAKALYANLEPKAEQ  
 YAVITWRALNKLPLVPQVANVVVPTAVYFSEKYNDVVRGTTEQGY  
 RVSSYLPLLPTEKITKVFGDEAS

## Additional Hevea sequences (NCBI entrez accession):

15 3319923; 3319921; 3087805; 1493836; 1480457; 1223884; 3452147;  
 3451147; 1916805; 232267; 123335; 2501578; 3319662; 3288200;  
 1942537; 2392631; 2392630; 1421554; 1311006; 494093; 3183706;  
 3172534; 283243; 1170248; 1708278; 1706547; 464775; 266892;  
 231586; 123337; 116359; 123062; 2213877; 542013; 2144920; 1070656;  
 20 2129914; 2129913; 2129912; 100135; 82026; 1076559; 82028; 82027;  
 282933; 280399; 100138; 1086972; 108697; 1086976; 1086978;  
 1086978; 1086976; 1086974; 1086972; 913758; 913757; 913756;  
 234388; 1092500; 228691; 1177405; 18839; 18837; 18835; 18833;  
 18831; 1209317; 1184668; 168217; 168215; 168213; 168211; 168209;  
 25 348137.

## Rye grass

Lolium sequences:

5

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15

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5.



**Olive tree****Olive sequences**

416610 Ole e 1

5 EDIPQPPVSQFHIQGQVYCDTCRAGFITELSEFIPGASLRLQCKDKEN  
GDVTFTEVGYTRAEGLYSMLVE  
RDHKNEFCEITLISSGRKDCNEIPTEGWAKPSLKFKLNTVNGTTTRTV  
NPLGFFKKEALPKCAQVYNKLG  
YPPNM

10

**Parietaria****Parietaria sequences:** \_\_\_\_\_

2497750 Par j P2

15 MRTVSMAALVVIAAALAWTSSAEPAPAPAPGEEACGKVVQDIMPC  
LHFVKGEEKEPSKECCSGTKKLSEEVKTTEQKREACKCIVRATKGIS  
GIKNELVAEVPKKCDIKTTLPITADFDCSKIQSTIFRGYY

1352506 Par j P5

20 MVRALMPCLPFVQGKEKEPSKGCCSGAKRLDGETKTGPQRVHACE  
CIQTAMKTYSDIDGKLVSEVPKHICGIVDSKLPPIDVNMDCKTVGVV  
PRQPQLPVSLRHGPVTGPSDPAHKARLERPQIRVPPPAPEKA

1532056 Par j P8

25 MRTVSMAALVVIAAALAWTSSAELASAPAPGEGPCGKVVHHIMPC  
LKFBKGEEKEPSKSCCSGTKKLSEEVKTTEQKREACKCIVAATKGIS  
GIKNELVAEVPKFCGTTTLPITADFDCSKIQSTIFRGYY

1532058 Par j P9

30 MRTVSAPSAVALVVIVAAGLAWTSLASVAPPAPAPGSEETCGTVVR

~~ALMPCLPFVQGKEKEPSKGCCSGAKRLDGETKTGLQRVHACECIQT~~  
 AMKTYSDIDGKLVSEVPKHCGIVDSKLPPIDVNMDCKTLGVVPRQP  
 QLPVSLRHGPVTGPSDBAHKARLERPQIRVPPPAPEKA

5 2497749 Par j P9

MRTVSARSSVALVVIVA AVLVTSSASVAPAPAGSEETCGTVVGA  
 LMPCLPFVQGKEKEPSKGCCSGAKRLDGETKTGPQRVHACECIQTA  
 MKTYSDIDGKLVSEVPKHCGIVDSKLPPIDVNMDCKTLGVLHYKG  
 N

10

1086003 Par j 1

MVRALMPCLPFVQGKEKEPSKGCCSGAKRLDGETKTGPQRVHACE  
 CIQTAMKTYSDIDGKLVSEVPKHCGIVDSKLPPIDVNMDCKTVGVV  
 PRQPQLPVSLRHGPVTGPSRSRPPTKHGWRDPRLEFRPPHRKKPNP

15 AFSTLG

Additional Parietaria sequences (NCBI entrez accession):

543659; 1836011; 1836010; 1311513; 1311512; 1311511; 1311510;

20 1311509; 240971.

Timothy grass

Phleum sequences:

25 Phl p 1

MASSSSVLLVVVLFVFLGSA YGIPKVPPGNITATYGDKWLDACS  
 TWYGKPTGAGPKDNGGACGYKDVDDDDDD

KKRGDEQKLRSA GFLELQFRRVKCKYPEG TKVTFHVEKGSNPNYL

30 ALLVKYVNGDGDVVAVDIKEKGKDKWIELKESWGAIWRIDTPDKL

GPFTVRYTTEGGTKTEAEDVIPEGWKADTSYESK

Phl p 1

MASSSSVLLVVALFAVFLGSAHGIPKVPPGPNITATYGDKWLDACS  
5 TWYGKPTAAGPKDNNGGACGYKDVDKPPFSGMTGCGNTPIFKSGRG  
CGSCFEIKCTKPEACSGEPVVVHITDDNEEPIAAYHFDLSGIAFGSM  
AKKGDEQKLRSAAGEVEIQFRRVKCKYPEGTKVTFHVEKGSNPNYL  
ALLVKFSGDGDVVAVDIKEKGKDKWIALKESWGAIWRIDTPEVLK  
GPFTVRYTTEGGTKARAKDVIPEGWKADTAYESK

10

Phlp 2

MSMASSSSSSLLAMAVLAALFAGAWCVPKVTFTVEKGSNEKHLAV  
LVKYEGLDTMAEVELREHGSDEWVAMTKGEGGVWTFDSEEPLOQP  
FNFRFLTEKGMKNVFDDVVPEKYTIGATYAPEE

15

Phl p 5

ADLGYGGPATPAAPAEAAPAGKATTEEQKLIEKINDGFKAALAAA  
AGVPPADKYKTFVATFGAASNKAFAEGLSAEPKGAAESSKAALTS  
KLDAAAYKLAYKTAEGATPEAKYDAYVATLSEALRIIAGTLEVHAV  
20 KPAAEEVKVIPAGELQVIEKVDSAFKVAATAANAAPANDKFTVFEA  
AFNNAIKASTGGAYESYKFIPALEAAVKQAYAAATVATAPEVKYTVF  
ETALKKAFTAMSEAQKAAKPATEATATATAAVGAATGAATAATG  
GYKV

25 Phl p 5

ADLGYGGPATPAAPAEAAPAGKATTEEQKLIEKINDGFKAALAAA  
AGVPPADKYKTFVATFGAASNKAFAEGLSAEPKGAAESSKAALTS  
KLDAAAYKLAYKTAEGATPEAKYDAYVATLSEALRIIAGTLEVHAV  
30 KPAAEEVKVIPAGELQVIEKVDSAFKVAATAANAAPANDKFTVFEA  
AFNNAIKASTGGAYESYKFIPALEAAVKQAYAAATVATAPEVKYTVF

ETALKKAITAMSEAQKAAKPATEATATATAAVGAATGAATAATGG  
YKV

Phl p 5b

5 AAAAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQ  
KLIEDINVGFKA AVAAAASVPAADKFKTFEAAFTSSSKAAAAKAPG  
LVPKLDAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEV  
HAVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAATAAATAPADDDKF  
TVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAPOV  
10 KYAVFEAALTKAITAMSEVQKVSQPATGAATVAAGAATTAAGAAS  
GAATVAAGGYKV

Phl p 5a

ADLGYGPATPAAPAAGYTPATPAAPAGADAAGKATTEEQKLIEKIN  
15 AGFKAALAGAGVQPADKYRTFVATFGPASNKAF AEGLSGEPKGAA  
ESSSKAALTSKLDAA YKLAYKTAEGATPEAKYDAYVATLSEALRII  
AGTLEVHAVKPAAEEVKVIPAGELQVIEKVDAAFKVAATAANAAP  
ANDKFTVFEAAFNDEIKASTGGAYESYKFIPALEAAVKQAYAATVA  
TAPEVKYTVFETALKKAITAMSEAQKAAKPAAAATATATAAVGAA  
20 TGAATAATGGYKV

Phl p 5

MAVQKYTVALFLAVALVAGPAASYAADAGYAPATPAAAGAEAGK  
ATTEEQKLIEDINVGFKA AVAAAASVPAADKFKTFEAAFTSSSKAA  
25 TAKAPGLVPKLDAAYSVSYKAAVGATPEAKFDSFVASLTEALRVIA  
GALEVHAVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAATAAATAP  
ADTVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAAP  
OVKYAVFEAALTKAITAMSEVQKVSQPATGAATVAAGAATTAAGAAS

Phl p 5

MAVQKYTVALFLAVALVAGPAASYAADAGYAPATPAAAGAEAGK  
 ATTEEQKLIEDINVGFKAAVAAAASVPAADKFKTFEAAFTSSSKAA  
 TAKAPGLVPKLDAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIA  
 5 GALEVHAVKPVTEDPAWPKIPAGELQIIDKIDAAFKVAATAAATAP  
 ADDKFTVFEEAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATV  
 AAAPQVKYAVFEAALTKAITAMSEVQKVSQPATGAATVAAGAATT  
 ATGAASGAATVAAGGYKV

10 Phl p 5

ADAGYAPATPAAAGAEAGKATTEEQKLIEDINVGFKAAVAAAASV  
 PAADKFKTFEAAFTSSSKAATAKAPGLVPKLDAAYSVAYKAAVGA  
 TPEAKFDSFVASLTEALRVIAGALEVHAVKPVTEEPGMAKIPAGEL  
 QIIDKIDAAFKVAATAAATAPADDDKFTVFEEAFNKAIKESTGGAYD  
 15 TYKCIPSLEAAVKQAYAATVAAAPQVKYAVFEAALTKAITAMSEV  
 QKVSQPATGAATVAAGAATTAAGAASGAATVAAGGYKV

Phl p 5

SVKRSNGSAEVHRGAVPRRGPRGGPGRSYAADAGYAPATPAAAGA  
 20 EAGKATTEEQKLIEDINVGFKAAVAAAASVPAADKFKTFEAAFTSS  
 SKAATAKAPGLVPKLDAAYSVAYKAAVGATPEAKFDSFVASLTEA  
 LRVIAGALEVHAVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAATAA  
 ATAPADDDKFTVFEEAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYA  
 ATVAAAPQVKYAVFEAALTKAITAMSEVQKVSQPATGAATVAAGA  
 25 ATTAAGAASGAATVAAGGYKV

Phl p 5

ATPAAPAGAEPAGKATTEEQKLIENAGFKAAALAAAAGVPPADKY  
 30 RTFVATFGAASNKAFAGLSGEPKGAAESSSKAALTSKLDAAAYKLA

YKTAEGATPEAKYDAYVATVSEALRIIAGTLEVHAVKPAAEEVKVI  
PAGELQVIEKVDAAFKVAATAANAAPANDKFTVFEAAFNDAIKAS  
TGGAYESYKFIPALEAAVKQAYAATVATAPEVKYTVFETALKKAIT  
AMSEAQKAAKPAAAATATATAAVGAATGAATAATGGYKV

5

Phl p 5

ADLGYGGPATPAAPAEAPAGKATTEEQKLIKINDGFKAALAAA  
AGVPPADKYKTFVATFGAASNKAFAEGLSAEPKGAAESSKAALTS  
KLDAAAYKLAYKTAEGATPEAKYDAYVATLSEALRIIAGTLEVHAV  
10 KPAAEEVKVIPAGELQVIEKVDSAFKVAATAANAAPANDKFTVFEA  
AFNNAIKASTGGAYESYKFIPALEAAVKQAYAATVATAPEVKYTVF  
ETALKKAFTAMSEAQKAAKPATEATATATAAVGAATGAATAATG  
GYKV

15 Phl p5b

AAAAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQ  
KLIEDINVGFKAAVAAAASVPAADKFKTFEAAFTSSSKAAAAKAPG  
LVPKLDAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEV  
HAVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAATAAATAPADDKF  
20 TVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAPQV  
KYAVFEAALTKAITAMSEVQKVSQPATGAATVAAGAATTAAGAAS  
GAATVAAGGYKV

Phl p5a

25 ADLGYGPATPAAPAAAGYTPATPAAPAGADAAGKATTEEQKLIEKIN  
AGFKAALAGAGVQPADKYRTFVATFGPASNKAFAGEGLSGEPKGAA  
ESSKAALTSKLDAAAYKLAYKTAEGATPEAKYDAYVATLSEALRIIAGTLEVHAV

AFNNAIKASTGGAYESYKFIPALEAAVKQAYAATVATAPEVKYTVFETALKKAITAMSEAQKAAKPAAAATATATAAVGAA

40-41  
C45  
TGAATAATGGYKV

Phl p 5

AVPRRGPRGGPGRSYAADAGYAPATPAAAGAEAGKATTEEQKLIE  
5 DINVGFKAAVAAAASVPAGDKFKTFEAAFTSSSKAATAKAPGLVPK  
LDAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVHAVK  
PVTEEPGMAKIPAGELQIIDKIDAAFKVAATAAATAPADDDKFTVFE  
AAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAPQVKYA  
VFEEALTKAITAMSEVQKVSQPATGAATVAAGAATTATGAASGAA  
10 TVAAGGYKV

Phl p 5b

MAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQKLI  
EDINVGFKAAVAARQRPAADKFKTFEAAASPRHPRPLRQGAGLVPKL  
15 DAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVHAVKP  
VTEEPGMAKIPAGELQIIDKIDAAFKVAATAAATAPADDDKFTVFEA  
AFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAAEVKYAV  
FEAALTKAITAMSEVQKVSQPATGAATVAAGAATTAAGAASGAAT  
VAAGGYKV

20

Phl p 5

MAVHQYTVALFLAVALVAGPAASYAADLGYGPATPAAPAAAGYTP  
ATPAAPAEAPAGKATTEEQKLIEKINAGFKAALAAAAGVQPADK  
YRTFVATFGAASNKAFAEGLSGEPKGAAESSSKAALTSKLDAAAYKL  
25 AYKTAEGATPEAKYDAYVATLSEALRIIAGTLEVHAVKPAAEEVKV  
IPAGELQVIEKVDAAFKVAATAANAAPANDKFTVFEAAENDAAVAS  
TGGAYFSYKEIPALF...  
...MSEVQKVSQPATGAATVAAGAATTATGAASGAA  
...TGAATAATGGYKV

30 Phl p 5

214  
5 EAPAGKATTEEQKLIKINAGFKAALARRLQPADKYRTFVATFGPA  
SNKAFAEGLSGEPKGAAESSKAALTSKLDAAAYKLAYKTAEGATPE  
AKYDAYVATLSEALRIIAGTLEVHAVKPAAEEVKVIPAAELQVIEKV  
DAAFKVAATAANAAPANDKFTVFEEAFNDEIKASTGGAYESYKFIP  
ALEAAVKQAYAATVATAPEVKYTVFETALKKAITAMSEAQKAAKP  
PPLPPPPQPPPLAATGAATAATGGYKV

Phl p 5

10 MAVHQYTVALFLAVALVAGPAASYAADLGYGPATPAAPAAGYTP  
ATPAAPAEAAPAGKATTEEQKLIKINAGFKAALAAAAGVQPADK  
YRTFVATFGAASNKAFAEGLSGEPKGAAESSKAALTSKLDAAAYKL  
AYKTAEGATPEAKYDAYVATLSEALRIIAGTLEVHAVKPAAEEVKV  
IPAGELQVIEKVDAAFKVAATAANAAPANDKFTVFEEAFNDAIKAS  
TGGAYESYKFIPALEAAVKQAYAATVATAPEVKYTVFETALKKAIT  
15 AMSEAQKAAKPAAAATATATAAVGAATGAATAATGGYKV

Phl p 5b

20 MAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQKLI  
EDINVGFKAAVAARQRPAADKFKTFEAAASPRHPRPLRQGAGLVPKL  
DAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVHAVKP  
VTEEPGMAKIPAGELQIIDKIDAAFKVAATAAATAPADDKFTVFEEA  
AFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAAEVKYAV  
FEAALTKAITAMSEVQKVSQPATGAATVAAGAATTAAGAASGAAT  
VAAGGYKV

25

Phl p 5a

ADLGYGPATPAAPAAGNTRATPA  
ESSKAALTSKLDAAAYKLAYKTAEGATPEAKYDAYVATLSEALRII  
30 AGTLEVHAVKPAAEEVKVIPAGELQVIEKVDAAFKVAATAANAAP



*Sub  
C17*

ANDKFTVFEEAFNDEIKASTGGAYESYKFIPALEAAVKQAYAATVA  
TAPEVKYTVFETALKKAITAMSEAQKAAKPPPLPPPQPPPLAATGA  
ATAATGGYKV

5 Phl p 5

MAVHQYTTVALFLAVALVAGPAASYAADLGYGPATPAAPAAGYTP  
ATPAAPAEAPAGKATTEEQKLIKINAGFKAALAAAAGVQPADK  
YRTFVATFGAASNKAFAEGLSGEPKGAAESSKAALTSKLDAA\_YKL  
AYKTAEGATPEAKYDAYVATLSEALRIIAGTLEVHAVKPAAEEVKV  
10 IPAGELQVIEKVDAAFKVAATAANAAPANDKFTVFEEAFNDAIKAS  
TGGAYESYKFIPALEAAVKQAYAATVATAPEVKYTVFETALKKAIT  
AMSEAQKAAKPAAAATATATAAVGAATGAATAATGGYKV

Phl p 6

15 MAAHKFMVAMFLAVAVVLGLATSPTAEGGKATTEEQKLIEDVNA  
SFRAAMATTANVPPADKYKTFEAAFTVSSKRNLADAVSKAPQLVP  
KLDEVYNAAYNAADHAAPEDKYEAFVLHFSEALRIIAGTPEVHAV  
KPGA

20 Phl p 6

SKAPQLVPKLDEVYNAAYNAADHAAPEDKYEAFVLHFSEALHIIAG  
TPEVHAVKPGA

Phl p 6

25 ADKYKTFEAAFTVSSKRNLADAVSKAPQLVPKLDEVYNAAYNAAD  
HAAPEDKYEAFVLHFSEALHIIAGTPEVHAVKPGA

TEEQKLIEDVNASFRAAMATTANVPPADKYKTLEAAFTVSSKRNL  
DAVSKAPQLVPKLDEVYNAAYNAAADHAAPEDKYEAFVLHFSEALR  
IIAGTPEVHAVKPGA

5

MAAHKFMVAMFLAVAVVLGLATSPTAEGGKATTEEQKLIEDINAS  
FRAAMATTANVPPADKYKTFEAAFTVSSKRNLADAVSKAPQLVPK  
LDEVYNAAYNAADHAAPEDKYEAFVLHFSEALHIIAGTPEVHAVK

10      PGA

MVAMFLAVAVVLGLATSPTAEGGKATTEEQKLIEDVNASFRAAMA  
TTANVPPADKYKTFEAAFTVSSKRNLADAVSKAPQLVPKLDEVYN  
AAYNAADHAAPEDKYEAFVLHFSEALRIIAGTPEVHAVKPGA

15

MADDMERIFKRFD TNGDGKISLSELTDA LR TLGSTADEVQRMMA  
EIDTDGDGFIDFNEFISFCNANPGLMKDVAKVF

20

MSWQTYVDEHLMCEIEGHHLASAAILGHDGTVWAQSADFPQFKPE  
EITGIMKDFDEPGHLAPTGMFVAGAKYMWIQGEPRVIRGKKGAG  
GITIKKTGQALVVGIIYDEPMTPGQCNMVVERLG DYLV EOGM

25

Additional Phleum sequences (NCBI entrez accession):

542167; 542168; 542169; 542171; 253337; 253336;  
453976; 439960.

*Dot  
219*  
Wasp (and related)

Vespula sequences:

5 465054 ALLERGEN VES V 5

MEISGLVYLIIVTHIDLPGKANNYCKIKCLKGGVHTACKYGSLKPN  
CGNKVVVS YGLTKQEKQDILKEHNDFRQKIARGLETRGNPGPQPPA  
KNMKNLVWNDELA YVAQVWANQCQYGHDTCDVAKYQVGQNV  
ALTGSTAAKYDDPVKLVKMWEDVKDYNPKKKFSGNDFLKTGHY  
10 TQMVWANTKEVGCGSIKIQEKWHKHVLCNYGPSGNFMNEELY  
QTK

1709545 ALLERGEN VES M 1

GPKCPFNSDTVSHIETRENRNRDL YTLQTLQNHPEFKKKTITRPVVF  
15 ITHGFTSSASEKNFINLAKALVDKDNMVISIDWQTA ACTNEYPGL  
KYA YPTAASNTRLVGQYIATITQKLVKDYKISMANIRLIGHSLGAH  
VSGFAGKRVQELKLGKYSEIIGLDPARPSFDSNHC SERLCETDAEYV  
QIIHTSNYLGTEKILGTVD FYMNNGKNNPGCGRFFSEVCSHTRAVIY  
MAECIKHECCLIGIPRSKSSQPISRCTKQECVCVGLNAKKYPSRGSFY  
20 VPVESTAPFCNNKGKII

1352699 ALLERGEN VES V 1

MEENMNLKYLLLFVYFVQVLNCCYGHGDPLSYELDRGPKCPFNSD  
TVSHIETRENRNRDL YTLQTLQNHPEFKKKTITRPVVFITHGFTSSAS  
25 ETNFINLAKALVDKDNMVISIDWQTA ACTNEAAGLKYLYPTAA  
RNTRLVGQYIATITQKLVKHYKISMANIRLIGHSLGAHASGFAGKKV  
QELKLGKYSEIIGI DPARPSFDSNHC SERLCETDAEYV  
CLIGIPKSKSSQPISSCTKQECVCVGLNAKKYPSRGSFYVPVESTAP  
30 FCNNKGKII

## 1346323 ALLERGEN VES V 2

SERPKRVFNIVWNVPTFMCHQYDLYFDEVTFNFIKRNSKDDFQGD  
 KIAIFYDPGEFPALLSLKDGKYKKRNGGVPQEGNITIHLQKFENLD  
 5 KIYPNRNFSGIGVIDFERWRPIFRQNWGNMKIHKNF SIDLVRNEHPT  
 WNKKMIELEASKRFEKYARFFMEETLKLAKKTRKQADWGYGYGP  
 YCFNMSPNNLVPECDVTAMHENDKMSWLFNNQNVLLPSVYVRQE  
 LTPDQRIGLVQGRVKEAVRISNNLKHSPKVLSYWWYVYQDETNTF  
 LTETDVKKTFQEI VINGGDGIIWGSSSDVNSLSKCKRLQDYLLTVLG  
 10 PIAINVTEAVN

## 549194 ALLERGEN VES VI

5KVNICKIKCLKGGVHTACKYGTSTKPNCGKMVVKAYGLTEAEK  
 QEILKVHNDFRQKVAKGLETRGNPGPQPPAKNMNNLVWNDELANI  
 15 AQVWASQCNYGHDTCKDTEKYPVGQNIKRSTTAALFDS PGKLVK  
 MWENEVKDFNPNIEWSKNNLKKTGHYTQM VWAKTKEIGCGSVKY  
 VKDEWYTHYLCNYGPSGNFRNEKLYEKK

Additional vespula sequences (NCBI entrez accession):

20 549193; 549192; 549191; 549190; 549189; 117414; 126761; 69576;  
 625255; 627189; 627188; 627187; 482382; 112561; 627186; 627185;  
 1923233; 897645; 897647; 745570; 225764; 162551.

Tree allergen sequences (mainly birch) sequences:

25

## 114922 Bet v 1

MGVFN YETETTSVIP AARLFKAFILDGDNI EPKVADQALGGVET  
 ...  
 ...  
 ...  
 30 ETLLRAVESYLLAHSDAYN

130975 Bet v 2

MSWQTYVDEHLMCDIDGQASNSLASAIVGHDGSVWAQSSSFPQFK  
PQEITGIMKDFEEPGHLAPTGLHLGGIKYMVIQGEAGAVIRGKKGSG  
5 GITIKKTGQALVFGIYEETVTPGQCNMVVERLGDYLIDQGL

1168696 Bet v 3

MPCSTEAMEKAGHGHASTPRKRSLSNSSFRLRSESLNTRLRLRRIFDL  
FDKNSDGHTVDELSRALNLLGLETDLSELESTVKSFTREGNIGLQFE  
10 DFISLHQS LNDSYFAYGGEDEDDNEEDMRKSILSQEEADSFGGFKV  
FDEDGDGYISARELQMVLGKLG FSEGSEIDRVEKMIVSVDSNRDGR  
VDFFEFKDMMRSVLRSS

809536 Bet v 4

15 MADDHPQDKAERERIFKRFDANGDGKISAAELGEALKTLGSITPDE  
VKHMMAEIDTDGDGFISFQEFTDFGRANRGLLKDVAKIF

543675 Que a I - Quercus alba = oak trees (fragment)

GVFTXESQETS VIAPAXLFLKALFL

20

543509 Car b I - Carpinus betulus = hornbeam trees (fragment)

GVFNIEAETPSVIPAAARLFKSYVLDGDKLIPKVAPQAIXK

543491 Aln g I - Alnus glutinosa = alder trees (fragment)

25 GVFNIEAETPSVIPAAARLFKAFILDGDKLLPKVAPEAVSSVENI

1204056 Bet v 2

MOVWDDI... QEAKEVDYLI RKNLIPCLL  
FELEHGFVYREHN RSPGYDGRYWTMWKLPMFGCNDSSQVLKEL

30 EECKKAYPSAFIRIIGFDDK

25.2

20

## Peanut sequences

25 MRGRVSPLMLLLGILVLASVSATHAKSSPYQKKTENPCAQRCLQSC  
QQEPDDLKQKACESRCTKLEYDPRCVYDPRGHTGTTNQRSPPGER  
TRGRQPGDYDDDRRQPRREEGGRWGPAGPRERFREEDWDBDRE  
WDDDSM  
26 KHADADNILVIQOGQATVTVANGNNRKSFNLDEGHALRIPSGEISYI

10 Ragweed

## Ambrosia sequences

113478 Amb a l

MGIKHCCYILYFTLALVTLLQPVRSAEDLQQILPSANETRSLTTCGT  
15 YNIIDGCWRGKADWAENRKALADCAQGFAKGTIGGKDGDIYTVTS  
ELDDDVANPKEGTLRFGAAQNRPLWIIFARDMVIRLDRELAINNDK  
TIDGRGAKVEIINAGFAIYNVKNIIHNIMHDIVVNPGGLIKSHDGPP  
VPRKGS DGAIGISGGSQIWIDHCSLSKA VDGLIDAKHGSTHFTVSN  
CLFTQH QYLLLFWD FDERGMLCTVAFNKFTDNVDQ RMPNLRHGF  
20 VQVVNN NYERWGSYALGGSAGPTILSQGNRFLASDIKKEVVGRYG  
ESAMSESINWNWRSYMDVFENG AIFVPSGVD PVLTPEQNAGMIPAE  
PGEAVLRLTSSAGVLSCOPGAPC

113479 Amb a 2

25 MGIKHCCYILYFTLALVTLVQAGRLGEEVDILPSPNDTRRSLQGCE  
AHNIIDKCWRCKPDWAENRQALGNCAQGFGKATHGGKWGDIYM  
VTSDQDDDVVNPKEGTIREGATQDDVHLL  
30 GVTISNCKFTTHEKAVLLGASDTHFQDLKMHVTLAYNIFTNTVHE

21/3/99

RMPCRFRGFFQIVNNFYDRWDKYAIGGSSNPTILSQGNKFVAPDFIY  
KKNVCLRTGAQEPEWMTWNWRTQNDVLENGAIFVASGSDPVLTA  
EQNAGMMQAEPGDMVPQLTMNAGVLTCSPGAPC

5 113477 Amb a 1.3

MGIKQCCYILYFTLALVALLQPVRS AEGVGEILPSVNETRSLQACEA  
LNIIDKCWRGKADWENNRQALADCAQGFAKGT YGGKWGDVYTV  
TSNLDDDDVANPKEGTLRFAAAQNRPLWIIFKNDMVINLNQELVVN  
SDKTIDGRGVKVEIINGGLTLMNVKNIIHNINIH DVKVLPGGMIKSN  
10 DGPPILRQASDGD TINVAGSSQIWIDHCSLSK SFDGLVDVTLGSTHV  
TISNCKFTQQSKAILLGADDTHVQDKGMLATVAFNMFTDNVDQR  
MPCRFRGFFQVVNNNYDRWGTYAIGGSSAPTILCQGNRFLAPDDQI  
KKNVLARTGTGAAESMAWNWRSDKD LLENGAIFVTSGSDPVLTPV  
QSAGMIPAEPGEAAIKLTSSAGVFSCHPGAPC

15

113476 Amb a 1.2

MGIKHCCYILYFTLALVTLLQPVRS AEDVEEFLPSANETRRLKACE  
AHNIIDKCWRCKADWANNRQALADCAQGFAKGT YGGKHGDVYT  
VTSDKDDDDVANPKEGTLRFAAAQNRPLWIIFKRN MVIHLNQELVV  
20 NSDKTIDGRGVKNIVNAGLTLMNVKNIIHNINIH DIKVCPPGMIKS  
NDGPPILRQQSDGDAINVAGSSQIWIDHCSLSK ASDGLLDITLGSSHV  
TVSNCKFTQH QFVLLL GADDTHYQDKGMLATVAFNMFTDHVDQR  
MPCRFRGFFQVVNNNYDRWGTYAIGGSSAPTILSQGNRFFAPDDIIK  
KNVLARTGTGNAESMSWNWRTDRD LLENGAIFLPSGSDPVLTP EQ  
25 KAGMIPAEPGEAVLRLTSSAGVLSCHQGAPC

113475 Amb a 1.1

MGIKQCCYILYFTLALVALLQPVRS AEGVGEILPSVNETRSLQACEA  
LNIIDKCWRGKADWENNRQALADCAQGFAKGT YGGKWGDVYTVTS  
ELDDDDVANPKEGTLRFGAAQNRPLWIIFERDMVIRLDKEMVVNSD



KTIDGRGAKVEIINAGFTLNGVKNVIIHNINMHDVKVNPGLIKSND  
 GPAAPRAGSDGDAISISGSSQIWIDHCSLSKSV DGLVDAKLGTTRLT  
 VSNSLFTQH QFVLLFGAGDENIEDRGMLATVAFNTFTDNVDQRM P  
 RCRHGFFQVVNNNYDKWGSYAIGGSASPTILSQGNRFCAPDERSKK  
 5 NVLGRHGEAAAESMKWNWRTNKDVLENGAIFVASGVDPVLTPEQ  
 SAGMIPAEPGESALSLTSSAGVLSCQPGAPC

### Cedar sequences

10 493634 Cry j IB precursor  
 MDSPCLVALLVFSFVIGSCFSDNPIDSCWRGDSNWAQNRMKLADC  
 AVGFGSSTMGGKGGDLYTVTNSDDDPVNPPGTLRYGATRDRPLWI  
 IFSGNMNIKLKMPMYIAGYKTFDGRGAQVYIGNGGPCVFIKRVS  
 IHHGLYLYGCSTSVLGNVLINESFGVEPVHPQDGDALTLRTATNIWI  
 15 DHNSFSNSSDGLVDVTLTSTGYTISNNLFFNHHKVMSLGHDDAYS  
 DKSMKVTVAFNQFGPNCGQRMPRARYGLVHVANNNYDPWTIYAI  
 GGSSNPILSEGNSFTAPNESYKKQVTIRIGCKTSSSCSNWVWQSTQ  
 DVFYNGAYFVSSGKYEGGNIYTKKEAFNVENG NATPHLTQNAGVL  
 TCSLSKRC

20 493632 Cry j IA precursor  
 MDSPCLVALLVLSFVIGSCFSDNPIDSCWRGDSNWAQNRMKLADC  
 AVGFGSSTMGGKGGDLYTVTNSDDDPVNPAPGTLRYGATRDRPL  
 WIIFSGNMNIKLKMPMYIAGYKTFDGRGAQVYIGNGGPCVFIKRVS  
 25 NVIIHGLHLYGCSTSVLGNVLINESFGVEPVHPQDGDALTLRTATNI  
 WIDHNSFSNSSDGLVDVTLSTGYTISNNLFFNHHKVMSLGHDDAYS  
 GGSSNPILSEGNSFTAPNESYKKQVTIRIGCKTSSSCSNWVWQST  
 QDVFYNGAYFVSSGKYEGGNIYTKKEAFNVENG NATPQLTKNAGV  
 30 LTCSLSKRC

1076242 Cry j II precursor - Japanese cedar

MAMKLIAPMAFLAMQLIIMAAAEDQSAQIMLDSVVEKYLRNRSRSL  
RKVEHSRHDAINIFNVEKYGAVGDGKHDCTEAFSTAWQAACKNPS  
5 AMLLVPGSKKFVVNNLFFNGPCQPHFTFKVDGIIAAYQNPASWKN  
NRIWLQFAKLTGFTLMGKGVIDGQGKQWWAGQCKWVNGREICND  
RDRPTAIKFDFSTGLIIQGLKLMNSPEFHLVFGNCEGVKIIIGISITAPR  
DSPNTDGIDIFASKNFHLQKNTIGTGDDCVAIGTGSSNIVIEDLICGP  
GHGISIGSLGRENSRAEVSYPVHVNGAKFIDTQNGRLRIKTWQGGSGM  
10 ASHIIYENVEMINSENPIINQFYCTASACQNQRSASVQIQDVTYKNI  
RGTSATAAAIQLKCSDSMPCCKDIKLSLKLTSGLIASCLNDNANG  
YFSGHVIPACKNLSPSAKRKESKSHKHPKTMVMVENMRAYDKGNRT  
RILLGSRPPNCTNKCHGCSPCKAKLVIVHRIMPQEYYPQRWICSCHG  
KIYHP

15

1076241 Cry j II protein - Japanese cedar

MAMKFIAPMAFVAMQLIIMAAAEDQSAQIMLDSIDIEQYLRNRSRLR  
KVEHSRHDAINIFNVEKYGAVGDGKHDCTEAFSTAWQAACKKPSA  
MLLVPGNKKFVVNNLFFNGPCQPHFTFKVDGIIAAYQNPASWKN  
20 RIWLQFAKLTGFTLMGKGVIDGQGKQWWAGQCKWVNGREICNDR  
DRPTAIKFDFSTGLIIQGLKLMNSPEFHLVFGNCEGVKNGISITAPRD  
SPNTDGIDIFASKNFHLQKNTIGTGDDCVAIGTGSSNIVIEDLICGPG  
HGISIGSLGRENSRAEVSYPVHVNGAKFIDTQNGRLRIKTWQGGSGMA  
SHIIYENVEMINSENPIINQFYCTASACQNQRSASVQIQDVTYKNIR  
25 GTSATAAAIQLKCSDSMPCCKDIKLSLKLTSGLIASCLNDNANGY  
FSGHVIPACKNLSPSAKRKESKSHKHPKTMVMVKNMGAYDKGNRTRI  
ILLGSRPPNCTNKCHGCSPCKAKLVIVHRIMPQEYYPQRWICSCHG  
KIYHP

30 541803 Cry j I precursor - Japanese cedar

MDSPCLVALLVLSFVIGSCFSDNPIDSCWRGDSNWAQNRMKLADC  
 AVGFGSSTMGGKGGDLYTVTNSDDDPVNPPTGLRYGATRDRPLWI  
 IFSGNMNIKLKMPMYIAGYKTFDGRGAQVYIGNGGPCVFIKRVS  
 IHHGLLYGCSTSVLGNVLINESFGVEPVHPQDGDALTLRTATNIWI  
 5 DHNSFSNSSDGLVDVTLSTGTISNNLFFNHHKVMLLGHDDAYSD  
 DKSMKVTVAFNQFGPNCGQRMPRARYGLVHVANNNYDPWTIYAI  
 GGSSNPILSEGNSFTAPNESYKKQVTIRIGCKTSSSCSNWVWQSTQ  
 DVFYNGAYFVSSGKYEGGNIYTKKEAFNVENG NATPQLTKNAGVL  
 TCSLSKRC

10

541802 Cry j I precursor- Japanese cedar

MDSPCLVALLVFSFVIGSCFSDNPIDSCWRGDSNWAQNRMKLADC  
 AVGFGSSTMGGKGGDLYTVTNSDDDPVNPAPGTLRYGATRDRPL  
 WIIFSGNMNIKLKMPMYIAGYKTFDGRGAQVYIGNGGPCVFIKRVS  
 15 NVIIHGLYLYGCSTSVLGNVLINESFGVEPVHPQDGDALTLRTATNI  
 WIDHNSFSNSSDGLVDVTLTSTGTISNNLFFNHHKVMSLGHDDAY  
 SDDKSMKVTVAFNQFGPNCGQRMPRARYGLVHVANNNYDPWTIY  
 AIGGSSNPILSEGNSFTAPNESYKKQVTIRIGCKTSSSCSNWVWQST  
 QDVFYNGAYFVSSGKYEGGNIYTKKEAFNVENG NATPHLTQNAGV  
 20 LTCSLSKRC

Dog

Canis sequences:

25 Can f l

MKTLLLTIGFSLIAILQAQDTPALGKDTVAVSGKWYLKAMTADQE  
 VPEKPDSVTPMILKAQKGGNI EAKITM TNGGGG  
 LQSQEAL EDEREFSRAKGLNQEILELAQSETCSPGGQ

30

*148*  
*288*

Serum albumin fragment

BAYKSEIAHRYNDLGEEHFRGLVL

Serum albumin fragment

5 LSSAKERFKCASLQKFGDRAFKAWSVARLSQRFPKADFAEISKVVT  
DLTKVHKECCHGDLLECADDRADLAKYMCENQDSISTKLKECCDK  
PVLEKSQCLAEVERDELPGDLPSLAADFVEDKEVCKNYQEAKDVF  
LGTFLYEYSRRHPEYSVSLLLRLAKEYEATLEKCCATDDPPTCYAK  
VLDEFKPLVDEPQNLVKTNCELFEKLGEYGFQNALLVRYTKKAPQ  
10 VSTPTLVVEVSRKLGKVGTKCCKKPESERMSCADDFLS

Can f 2

MQLLLLTVGLALICGLQAQEGNHEEPQGGLEELSGRWHSVALASN  
KSDLIKPWGHFRVFIHMSAKDGNLHGDILIPQDGQCEKVSLTAFKT  
15 ATSNKFDLEYWGHNDLYLAEVDPKSYLILYMINQYNDTSLVAHL  
MVRDLRSRQQDFLPAFESVCEDIGLHKDQIVVLSDDDRCQGSRD

Additional dog allergen protein (NCBI entrez accession):

20 1731859

Horse

Equus sequences:

25 1575778 Equ c1

MKLLLLCLGLILVCAQQEENSVAIRNFDISKISGEWYSIFLASDVF  
EKIDENGCM...  
EDGVN...  
REPDVSPKEEFVKIVQKRGIVKENIIDLTAKIDRCFQLRGNGVAQA

30

3121755 Equ c 2

SQXPQSETDYSQLSGEWNTIYGAASNIXK

5 Euroglyphus (mite)

Euroglyphus sequences:

Eur m 1 (variant)

TYACSINSVSLPSELRLSLRTVTPIRMQGGCGSCWAFSGVASTESA  
10 YLAYRNMSLDLAEQELVDCASQNGCHGDTIPRGIEYIQNGVVQE  
HYPPYVAREQSCHRPNAQRYGLKNYCQISPPDSNKIRQALTQTHTA  
VAVIIGIKDLNAFRHYDGRTIMQHDNGYQPNYHAVNIVGYGNTQG  
VDYWIVRNSWDTTWGDNGYGYFAANINL

15 Eur m 1 (variant)

TYACSINSVSLPSELRLSLRTVTPIRMQGGCGSCWAFSGVASTESA  
YLAYRNMSLDLAEQELVDCASQNGCHGDTIPRGIEYIQNGVVQE  
HYPPYVAREQSCHRPNAQRYGLKNYCQISPPDSNKIRQALTQTHTA  
VAVIIGIKDLNAFRHYDGRTIMQHDNGYQPNYHAVNIVGYGNTQG  
20 VDYWIVRNSWDTTWGDNGYGYFAANINL

Eur m 1 (variant)

ETNACSINGNAPAEIDLRQMRTVTPIRMQGGCGSCWAFSGVAATES  
AYLAYRNQSLDLAEQELVDCASQHGCHGDTIPRGIEYIQHNGVVQE  
25 SYYRYVAREQSCRRPNAQRFGISNYCQIYPPNANKIREALAQTHSAI  
AVIIGIKDLDAFRHYDGRTHIQRDNGYQPNYHAVNIVGYSNAOGVD

Eur m 1 (variant)

30 ETSACRINSVNPSELRLSLRTVTPIRMQGGCGSCWAFSGVAATES

2001  
C 30

AYLAYRNTSLDLSEQELVDCASQHGCHGDTIPRGIEYIQQNGVVEE  
RSYPYVAREQQCRRPNSQHYGISNYCQIYPPDVKQIREALTQTHTAI  
AVNGIKDLRAFQHYDGRTHIQHDNGYQPNYHAVNIVGYGSTQGVD  
YWIVRNSWDTTWGDSDGYGYFQAGNNL

5

Poa (grass) sequences

113562 POLLEN ALLERGEN POA P 9

MAVQKYTVLFLVALVVGPAASYAADLSYGAPATPAAPAAGYTP  
10 AAPAGAAPKATTDEQKMKIEKINVGFKA AVAAAGGVPAANKYKTFV  
ATFGAASNKAFAEALSTEPKGAAVDSSKAALTSKLDAAAYKLAYKS  
AEGATPEAKYDDYVATLSEALRIIAGTLEVHGVKPAAEEVKATPAG  
ELQVIDKVDAAFKVAATAANAAPANDKFTVFEEAFNDAIKASTGG  
AYQSYKFIPALEAAVKQSYAATVATAPAVKYTVFETALKKAITAMS  
15 QAQKAAKPAAAATGTATAAVGAATGAATAAAGGYKV

113561 POA P 9

MAVHQYTVALFLAVALVAGPAASYAADVGYGAPATLATPATPAA  
PAAGYTPAAPAGAAPKATTDEQKLIKINAGFKA AVAAAAGVPAV  
20 DKYKTFVATFGTASNKAFAEALSTEPKGAAAASSNAVLTSKLDAA  
YKLAYKSAEGATPEAKYDAYVATLSEALRIIAGTLEVHAVKPAGEE  
VKAIPAGELQVIDKVDAAFKVAATAANAAPANDKFTVFEEAFNDA  
IKASTGGAYQSYKFIPALEAAVKQSYAATVATAPAVKYTVFETALK  
KAITAMSQAQKAAKPAAAVTATATGAVGAATGAVGAATGAATAA  
25 AGGYKTGAATPTAGGYKV

113560 POA P 9

ADKANGAYETALN...  
VGFAKKLDAFIQTSYLSSTKAAEPKEKFDLFLVLSLTEVLRFMAGAVK  
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AAVAAAAGVPAASKYKTFVATFGAASNKAFAEALSTEPKGAAVAS  
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 LEVHGVKPAAEVKAIPAGELQVIDKVDAAFKVAATAANAAPAND  
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 5 AVKYTVFETALKKAITAMSQAQKAAPAAVTGTATSAVGAATGA  
 ATAAAGGYKV

### Cockroach sequences

10 2833325 Cr p1

MKTALVFAAVVAFVAARFPDHKDYKQLADKQFLAKQRDVLRLFH  
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 RGEPFSVNYEKHREQAIMLYDLLYFANDYDTFYKTACWARDRVN  
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 15 KYWMKNAGSGEHHSHVIPVNFTLRTQDHLLAYFTSDVNLNAFNTY  
 YRYYPYPSWYNTTLYGHNIDRRGEQFYTYTKQIYARYFLERLSNDLP  
 DVYPPFYYSKPVKSAYNPNLRYHNGEEMPVRPSNMYVTNFDLYYIA  
 DIKNYEKRVEDAIDFGYAFDEHMKPHSLYHDVHGMEYLADMIEG  
 NMDSPNFYFYGSIYHMYHSMIGHIVDPYHKMGLAPSLEHPETVLR  
 20 DPVIFYQLWKRVDHLFQKYKNRLPRYTHIDELAFEGVKVENVDVGK  
 LYTYFEQYDMSLDMAVYVNNVDQISNVDVQLAVRLNHPFTYNIE  
 VSSDKAQDVYVAVFLGPKYDYLGREYDLNDRRHYFVEMDRFPYH  
 VGAGKTVIERNSHDSN<sup>1</sup>APERDSYRTFYKKVQEAYEGKSQYYVDK  
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 25 KAFSYCGVGSEKYPDNKPLGYPFDRKIYSNDFYTPNMYFKDVIIF

2231297 Cr p2

INEIHSIIGLPPFVPPSRRHARRGVGINGLIDDVIAILPVDELKALFQE  
 KIETSPDFKAIYDAIRSFEEQHSSTANMQRMHMQNIRDKGVNDVD

248  
392  
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5 RVDQGTLRRTLSSGQRNLQDDLNDLALIPTDQILAIAMDYLANDAE  
VQELVAYLQSDDFHKKIITTIEALPEFANFYNFLKEHGLDVVDYINEI  
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10 1703445 Bla g 2

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NVQVKFFDTGSAVGRGIEDSLTISNLTTSSQQDIVLADELSQEVCLISA  
DVVVGIAAPGCPNALKGKTVLENFVEENLIAPVFSIHHARFQDGEH  
15 FGEIIFGGSDWKYVDGEFTYVPLVGDDSWKFRLDGVKIGDTTVAPA  
GTQAIIDTSKAIIVGPKAYVNPINEAIGCVVEKTTTRICKLDCSKIPS  
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VDHYYSEFNWENKTMGFGRSVE  
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20

1705483 Bla g 4

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Additional cockroach sequences (NCBI Entrez accession numbers):

- 5 2580504; 1580797; 1580794; 1362590; 544619; 544618; 1531589;  
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Allergen (general) sequences:

NCBI accession numbers

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Example 7: Desensitisation using multiple overlapping peptides  
25 (MOP) from Fel d I

We have obtained data with multiple

peptides. These are three FCIP  
peptides. Originally, 10 peptides spanning both chain 1 and chain 2 of the  
30 Fel d I molecule were designed in order to increase the percentage of

individuals reacting to the peptide injection. By using peptides covering the entire molecule, we believed that we would cover more MHC-peptide pairings and thus get more reactors. Of the 16 peptides, the first three of chain 2 displayed poor solubility in aqueous solution and were excluded from the *in vivo* preparation termed MOP. The sequences of the MOP peptides and how they relate to the parent molecule are given in Figure 9.

We have carried-out a dose ranging study with this preparation to determine an appropriate dose to be used in a planned clinical trial in which four injections of increasing dose will be given over a two week period. For the dose ranging study, three doses have been tested: 1 $\mu$ g (of each of the 13 peptides in a mixture), 2.5 $\mu$ g and 5 $\mu$ g.

Four cat asthmatic individuals received the 1 $\mu$ g dose. One of them developed a LAR which was similar to those induced with FC1P. Five individuals received 2.5 $\mu$ g and again one developed a LAR. At 5 $\mu$ g, eight individuals were tested and four developed a LAR. This demonstrates the dose response effect that we expected and, more importantly, shows that the MOP preparation produces a similar effect to the FC1P preparation. An example of a LAR induced by MOP can be seen in Figure 10.

Rather than move to a higher dose which may give a higher percentage of LAR reactors, we have decided to use the 5 $\mu$ g dose as the starting dose for the trial. From the number of peptides in the MOP preparation and the observed dose response, it might be expected that some of the non-LAR reactors at 5 $\mu$ g might develop a LAR at a higher dose, ie have the appropriate MHC molecules.

We have decided to investigate the late phase reaction to whole allergen extract as an alternative clinical outcome. Basically, if whole allergen extract is injected

intradermally (in our case into the forearm) in an atopic allergic individual, an immediate wheal and flare reaction will result (classical IgE mediated early allergic reaction) in about 15 minutes. This reaction is then followed by a delayed in-time phase reaction in the skin. Like the  
5 lung reaction, this peaks at 6-9 hours and believed to be driven at least in part by T cells.

Previously, immunotherapy studies using conventional whole allergen extract have demonstrated that the size of this late phase skin reaction  
10 decreases after several months of treatment.

We have measured these skin reactions before any peptide injection (ie at baseline) and we have measured them again in six patients (to date) who have had either one or two injections only of MOP. All six have reduced  
15 reactions as shown in figure 11. These results are statistically significant with a p value of 0.036.

A further interesting observation was that some of these individuals did not develop a lung reaction (LAR) to the MOP injection but clearly their T  
20 cells were activated by one or more of the peptides giving them a measurable reduction in reactivity to skin challenge with whole allergen extract (the latter being perhaps even more significant since the whole dander extract contains multiple proteins (including Fel d I) to which the patient may be sensitised).

25

As mentioned above, some (three) of the MOP injected individuals who developed lung reactions have received a second injection. As found with FC1P, these individuals did not develop reactions to the second injection (in figure 10). These individuals received the second injection (in our case about 4 months) after the first one.

This suggests that hyporesponsiveness induced after the first injection could last four months or more.

We also have other longitudinal data regarding the length of duration of the hyporesponsiveness from some of the FC1P patients. In this case, three patients who had received FC1P more than one year ago and experienced LAR's were rechallenged with the same dose. All three reacted with almost exactly the same magnitude as the initial reaction (Figure 12a, b & c). Of these three, one (Figure 12a) had received a second injection of FC1P a few weeks after the first and had displayed no LAR. Thus, peptides can induce a LAR which is followed by hyporesponsiveness which seems to last for four months (possibly more) but less than one year.

Finally, we now have three FC1P patients who have had one injection followed by a LAR which on reinjection was not seen (ie hyporesponsiveness). We also have the same finding in two MOP patients. We have had the areas under these curves analysed statistically.

We have compared a control day (either saline injection or injection or whole cat extract, the latter does not induce a lung reaction only a skin reaction at the dose used), with the lung measurements (FEV1) after the first FC1P or MOP injection and after the second FC1P/MOP injection.

We have compared the mean values from spirometry by area under the curve analysis:

1. Control day vs peptide day 1 (we expect to see a significant difference since lung responses are back to normal)
2. Peptide day 1 vs peptide day 2 (expect a significant difference)
3. Peptide day 1 vs peptide day 2 (expect a significant difference)



The results (p values) are:

1.  $p=0.0205$
2.  $p=0.0930$
- 5 3.  $p=0.0119$

A p value of less than or equal to 0.05 is considered statistically significant.

- 10 Thus, there is a significant response to the peptides following the first injection (1) which is significantly different to the second injection (3) as the FEVI values appear to return to baseline. The difference between the control day and the second injection is not statistically significant (2).